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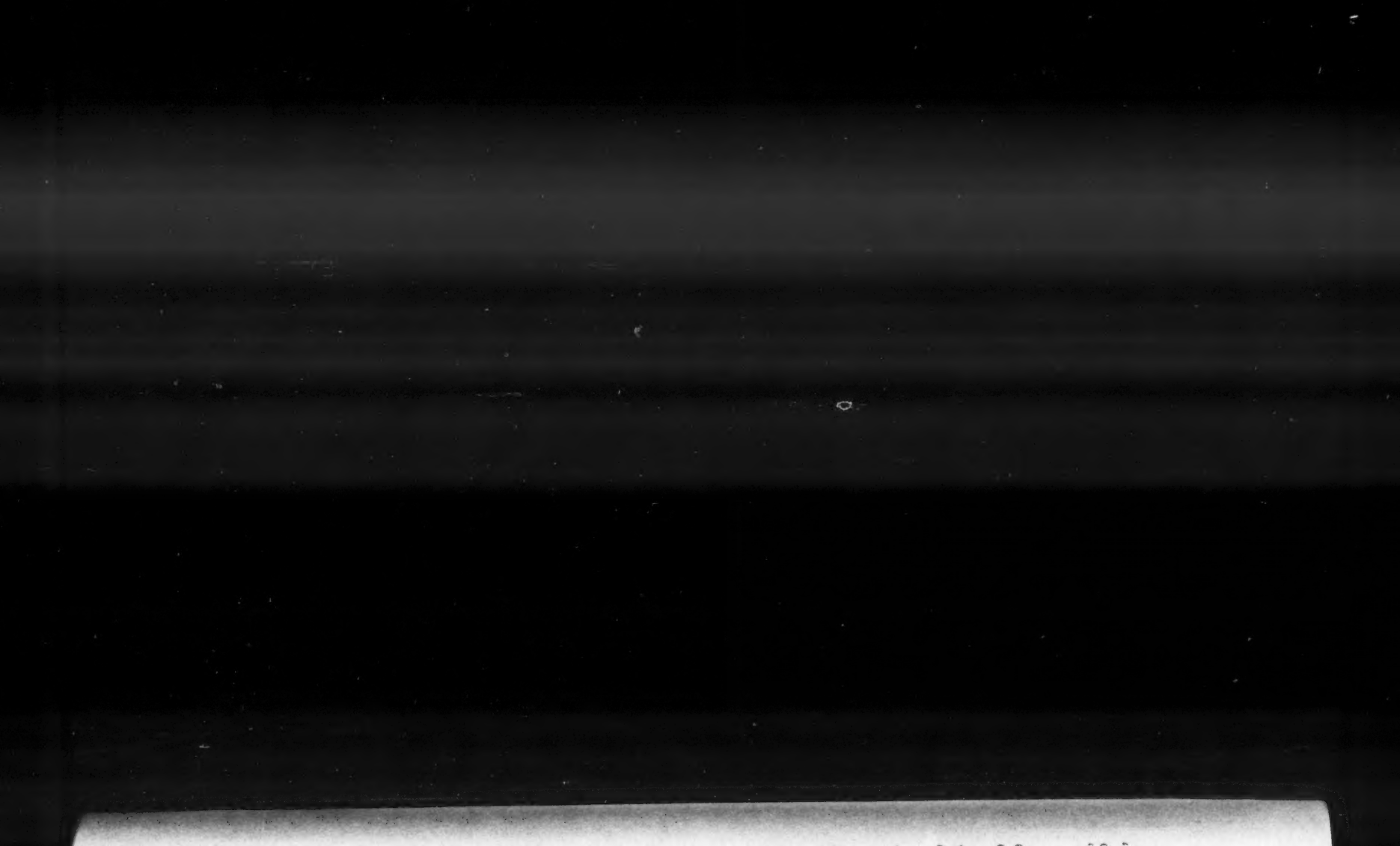

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JULY, 1930

EFFECTS OF INSOLATION AND SOIL CHARACTERISTICS ON TISSUE FLUID REACTION IN WHEAT

W. F. Loehwing

(WITH ONE FIGURE)

Introduction

In an earlier investigation of certain grain plants grown on humus soils, foliar chlorosis was frequently observed following use of lime to correct soil acidity (**31**). Tissue fluids of plants from alkalinated humus soils were found to be uniformly low in free acidity, and iron chlorosis was observed in plants in the low acid ranges. The prevalence and increasing severity of chlorosis during continued periods of intense insolation led to the investigation described herein to determine the effect of light on the acidity of sap, and the mobility of iron, in wheat. A number of investigators (**9, 13, 14, 23, 53**) have shown that the acidity of tissue fluids may decrease in light but there is as yet no wholly reliable evidence that this diminution in acidity during ordinary daylight is sufficient to interfere with the solubility and translocation of iron. In fact, it appears that under optimal conditions of nutrition, light does not always appreciably diminish the acidity of non-succulents **24, 46**). The correlation of strong light with chlorosis in plants grown on humus soils low in mineral content, however, suggested that under such conditions the effects of insolation might be sufficiently accentuated to cause injury.

Methods

Two sets of Marquis wheat were grown in a greenhouse at 20° C. One set of plants was grown on a strongly acid humus, low in mineral matter, and another set on a fertile but acid loam. Approximately half of the soil in each set was treated with 2000 parts per million of powdered calcium carbonate. When the plants were six weeks old a thin, white muslin screen was fastened above half of the plants in both the limed and unlimed series in order to reduce the intensity of the illumination. The remaining cul-

tures were left exposed to direct light. Though old and young leaves displayed differences in the degree of acidity, the general trend of hydrion variations under comparable conditions was consistently the same in all parts of the tops. This fact made it feasible to analyze the sap expressed from entire tops without risk of obliterating significant differences among the tissue fluids of variously treated plants.

Entire tops of several plants in each set were cut at consecutive four-hour intervals. These tops were immediately placed in large stoppered vials and frozen at $-30°$ C. Tissues were cut in a Nixtamal mill and the sap was then expressed from thirty gram samples under uniform pressure in a chilled steel press. Duplicate potentiometric determinations by means of the calomel half cell and quinhydrone electrode method (**8**) were rapidly made at 20° C. on 5-cc. aliquots. Three series of hydrion measurements are reported. The first analyses (table I) were started on the morning the muslin screen was placed over a portion of the six week old cultures. The second series of measurements (table II) was made on the sap of eight week old plants following a period of strong insolation during which symptoms of iron chlorosis had become conspicuous in the younger leaves of plants on the limed humus. Moisture content of the fresh tops of eight week old plants varied between eighty-four and eighty-eight per cent. of the fresh weight. The final analyses (table III) show the acidity and moisture content of ten week old chlorotic plants.

Though freezing and maceration of tissues to some extent alter the pH value of expressed sap this treatment was found to give more uniformity in the acidity determinations of aliquots from similar cultures than in sap expressed from unfrozen tissues (**35**). No attempt was made to eliminate solids suspended in the sap because of negligible differences between centrifuged and uncentrifuged aliquots. The difficulty in keeping samples chilled while centrifuging and the time consumed in the process caused greater pH fluctuations than those due to solids in the highly fluid type of saps obtained.

Determination of acidity in sap expressed by pressure has been criticized by REA and SMALL (**44**) because it furnishes no very obvious clues as to the processes by which it is brought about. Investigation of expressed sap has not, however, proven entirely fruitless because the degree of acidity has been found to be measurably correlated with illumination (**9, 12, 14, 23, 46, 51, 53**), the photoperiod (**13**), temperature (**3, 26**), age (**3, 4**), season (**1, 22**), the axial gradient (**11, 28**), and nutrient conditions (**6, 7, 10, 31, 36**). Discovery of such correlations is often the initial step in the determination of their underlying physiological mechanisms. Aided by knowledge of hydrion ranges and buffer indices in certain plants, MARTIN

(**32, 33, 34**), by a study of expressed sap, has very effectively identified important components of the plant buffer mechanism.

Difficulty in differentiating tints seriously limits the utility of intravital staining methods (**27, 39, 50**). These methods involve a definitely circumscribed technique in order to secure the penetration of indicators without cell injury (**27**). SMALL (**50**) and his collaborators have shown that the cell wall, cytoplasm and vacuole of a cell may differ distinctly in acidity. Consequently, objections have arisen to the customary usage of the expression "cell acidity" (**55**). Intravital methods of studying the tissue acidity of plants have not yet surpassed the simpler methods employing expressed sap to determine the causes of changes in reaction.

Discussion

All plants used for the initial acidity determinations (table I) during the sixth week of the experiment were in the tillering stage and apparently healthy in appearance. At the time of the second series of acidity determinations during the eighth week (table II) the majority of the plants on loam soil had ceased tillering and were rapidly elongating. Shaded plants of the same age on the humus soil approached the foregoing in size but not in erectness, vigor or depth of color. Humus plants receiving full illumination lagged behind in stature and maturation. Incipient chlorosis appeared among the unshaded plants on the limed humus soil during the eighth week. Some evidences of chlorosis among the shaded plants on this soil developed about ten days later but at no time became as frequent or severe as among the fully illuminated plants.

The data show that young wheat responded quickly to environmental changes. Fluctuations in sap acidity definitely reflected changes in soil reaction and light conditions. The free acidity of expressed tissue fluids of plants harvested at consecutive four-hour intervals disclosed a regular diurnal cycle with an early morning maximum and a late afternoon minimum in all cultures. The daily periodicity in hydrion concentration may be graphically depicted as a V-shaped curve whose left limb represents the fall from morning maximum and whose right limb represents the subsequent nocturnal rise. Similar decreases in sap acidity during periods of daylight have been reported by HAAS (**14**) and by TRUOG and MEACHAM (**53**) for corn, by GARNER (**13**) and his collaborators for soy-beans, by CLEVENGER (**9**) for cowpeas, and by HURD (**23, 25**) for wheat. HURD, however, states that normally the daily sap hydrion fluctuations are insignificant compared to those induced by maturation processes.

The above observations suggest the well-known diurnal hydrion periodicity in succulents due to acid photolysis in light (**12, 45, 51, 52**). Photo-

TABLE I

SAP HYDRION CONCENTRATION (pH) OF SAP EXPRESSED FROM SIX WEEK OLD WHEAT PLANTS

HOUR CUT	TYPE OF ILLUMINATION	HUMUS SOIL		LOAM SOIL	
		UNLIMED	LIMED	UNLIMED	LIMED
6 A. M.	Shaded	5.47	6.22	5.67	6.20
	Full	5.47	6.22	5.67	6.20
10 A. M.	Shaded	5.51	6.28	5.68	6.42
	Full	5.57	6.42	5.68	6.42
2 P. M.	Shaded	5.55	6.30	5.69	6.44
	Full	5.62	6.51	5.70	6.46
6 P. M.	Shaded	5.59	6.38	5.70	6.46
	Full	5.69	6.63	5.74	6.51
10 P. M.	Shaded	5.55	6.40	5.69	6.45
	Full	5.64	6.62	5.71	6.51
6 A. M.	Shaded	5.49	6.18	5.64	6.17
	Full	5.50	6.18	5.65	6.22

sensitive carboxylic acids have been reported in certain non-succulents (**34, 50**) and they may be rather widely distributed in small amounts. It is thus possible that slight reductions in the sap acidity of non-succulents in light may also be ascribable to acid photolysis. This daily acid rhythm may also be caused in part by the assimilation of some component of the buffer system during the normal metabolism of the plant. No definite information is as yet available as to the nature of the variable component of the sap responsible for its daily pH fluctuations. Although SAYRE (**47**) and SCARTH (**48, 49**) have shown that rather small variations in the sap acidity of guard cells are peculiarly important in the regulation of stomatal apertures, it is doubtful if the small daily fluctuations in the water content of leaves measurably influence acid periodicity of entire tops (**29, 30, 47**). Daily acid periodicity does not, however, appear to be as wide-spread nor as accentuated in non-succulents as in cacti.

On account of their obvious importance, studies on the photosynthetic and tropic effects of light on plants rightly hold a preëminent place in physiological research. Much recent work, however, indicates that light is directly important to plants in a number of other ways. Investigation of these less obvious effects of light would probably clarify many plant processes and might incidentally reveal the mechanism of diurnal acid

TABLE II

SAP HYDRION CONCENTRATION (pH) OF SAP EXPRESSED FROM EIGHT WEEK OLD WHEAT PLANTS

HOUR CUT	HUMUS SOIL				LOAM SOIL	
	UNLIMED		LIMED		UNLIMED	LIMED
	UNSHADED	SHADED	*UNSHADED	SHADED	UNSHADED	UNSHADED
6 A. M.	5.55	5.47	6.12	6.08	5.84	6.03
10 A. M.	5.59	5.50	6.31	6.18	5.89	6.07
2 P. M.	5.67	5.54	6.42	6.36	5.96	6.11
6 P. M.	5.71	5.58	6.51	6.40	5.99	6.24
10 P. M.	5.85	5.61	6.54	6.40	5.97	6.26
2 A. M.	5.57	5.54	6.38	6.20	5.92	6.12
6 A. M.	5.59	5.49	6.22	6.17	5.89	6.05
10 A. M.	5.65	5.52	6.30	6.19	5.93	6.08
2 P. M.	5.72	5.56	6.51	6.35	5.98	6.16
6 P. M.	5.79	5.56	6.67	6.35	6.03	6.31
10 P. M.	5.72	5.54	6.67	6.28	6.01	6.26
6 A. M.	5.50	5.61	6.25	6.21	5.90	6.10
10 A. M.	5.67	5.55	6.38	6.28	5.96	6.17

* Plants of this series showed incipient chlorosis.

periodicity in non-succulent plants. The investigations of WEISMANN (**56, 57**), NĚMEC and GRACANIN (**37**), HOAGLAND and DAVIS (**21**) and WLODEK (**58**) suggest that light more or less directly influences the intake of mineral nutrients by plants. Certain catalytic but non-photolytic effects of light observed by PRIESTLEY (**41**) and others (**5, 43, 54**) profoundly affect plant development. In addition to the above, there are direct effects of light on enzymatic activity (**38**) and the photocapillary effect described by HERČÍK (**15, 16, 17**).

It will be noted (fig. 1) that fluctuations occur from day to day in the hour and level of maximal and minimal acidity but the rhythmic rise and fall continued until the end of the experiment in all except the most chlorotic plants. The variation in the time and degree of the afternoon minimal acidity was, however, consistently greater than the variation in the morning maxima. The steepness in slope of the acidity curve appears to be determined primarily by the hour and level of the afternoon minimum acidity.

The fact that reversals in slope of the diurnal acidity gradient are not coincident with the hour of maximal light intensity suggests that the thermal factor in insolation may not be as important as its light effect. Though no attempt was made to measure foliar temperature, spectral

composition, or thermal energy of the light, it is certain that the thermal maxima of the plants were reached much earlier in the afternoons than the maximum pH of the sap. HURD-KARRER (**26**) has shown that sap hydrion concentration in wheat rises with the temperature. Thermal and light stimuli thus appear to exert opposite effects on the acidity of tissue fluids in wheat. The fact that sap pH increased in light despite the probable concomitant rise in leaf temperature further suggests the predominance of the photo-effect. It remains a matter for further investigation, however, to determine what portion of the diurnal hydrion variation herein reported is ascribable to temperature increments of the tissues and what fraction directly to light.

Though illumination uniformly diminished sap acidity as described above, differences in light intensity and soil alkalinity exercised considerable influence upon the level of acidity attained. Limed cultures in the shaded and unshaded sets maintained a lower degree of acidity and exhibited a greater initial decrease in sap hydrion concentration than the corresponding untreated plants exposed to full light intensity. In fact, the pH values of the sap from the limed cultures exposed to strong insolation for several consecutive days (LL, fig. 1) suggests that the period of darkness is too brief for complete acid recovery. Hence acidity falls progressively to lower levels and shows smaller diurnal fluctuations during prolonged periods of strong illumination. Microchemical analyses of leaves show that the diminished acidity of the sap increasingly interferes with iron mobility and finally induces chlorosis such as became apparent in the eight week old plants on the limed humus.

The onset of chlorosis in young leaves was delayed and was less severe in plants which were shaded following the use of lime. This fact, together with the pH values of these plants, suggests that shading to a considerable extent offsets the iron insufficiency created by lime. The acid recovery in shaded lime cultures (SL, fig. 1) does not, however, reach the original level of acidity found in the sap of plants grown on the untreated humus (LU, fig. 1). Microchemical inspection of chlorotic plants disclosed an abundance of iron in the roots but little or none in young leaves.

Considerable interest attaches to the fact that the most vigorous plants grew on the loam soil and the poorest on the fully illuminated, untreated and limed humus. Treated and untreated plants in this soil, though both low in vigor and retarded in development, represent opposite extremes in sap acidity. Seeds in the untreated humus germinated rapidly and the seedlings to all outward appearances grew normally until the fifth week. The rate of stem elongation then diminished and the leaves remained narrow though increasing in length. During the eighth week the older leaves turned a dull green while their tips became flaccid and turned brown.

The leaves then gradually died back toward the stem. The foregoing symptoms differed from those displayed by plants on the same soil after it had been limed. In the latter instance new leaves became noticeably chlorotic during the eighth week, and the severity of this condition increased with age. Reduced size and delay in maturation marked both the limed and untreated plants on the humus soil. Thus the hyperacidity of the plant on untreated humus on the one hand and the alkalinity of the strongly insolated, limed plants on the other, appeared equally injurious to wheat in its formative stages.

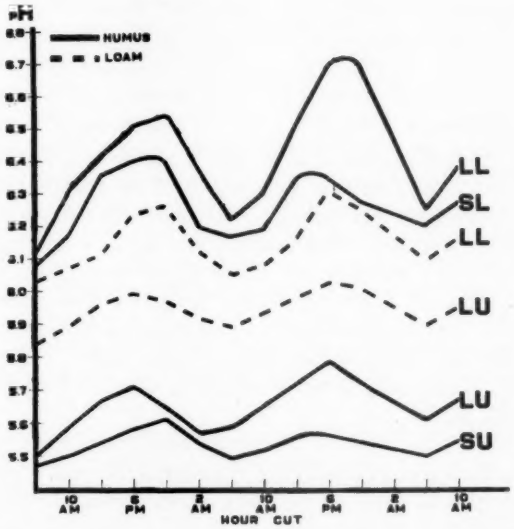

FIG. 1. Diurnal variation of free acidity (pH) in sap expressed from entire tops of eight week old wheat plants. LL, unshaded plants on limed soil; SL, shaded plants on unlimed soil; LU, unshaded plants on unlimed soil; SU, shaded plants on unlimed soil.

During the tenth week, it was found that mature leaves of fully illuminated, chlorotic plants on the limed humus gradually began to lose their turgor and turn brown. Many of the oldest leaves wilted and died. Acidity tests made on these plants during the incipient stages of browning (table III) disclosed that the sap hydrion concentration had risen considerably above that found in the eight week old plants of the same series. Moisture content had also fallen about five per cent. Diurnal acid periodicity could no longer be detected and the hydrion level approximated that of plants growing on the untreated acid humus. This latent increase

TABLE III

SAP HYDRION CONCENTRATION OF FULLY ILLUMINATED, CHLOROTIC PLANTS (TEN WEEKS OLD) ON LIMED HUMUS

HOUR CUT	pH
6 A. M.	5.57
10 A. M.	5.55
2 P. M.	5.59
6 P. M.	5.59
10 P. M.	5.62
6 A. M.	5.58
10 A. M.	5.55
2 P. M.	5.55
6 P. M.	5.58

in the acidity of initially low-acid chlorotic plants is probably due to the accumulation of acid catabolic products and to the decrease in tissue fluids. The entire symptom complex of these plants reflects an incoordination of metabolic processes which finally culminated in acid toxicity. Thus the extremes of high and low soil acidity both eventually result in sap hyperacidity. Though acidity generally increases with rises in temperature, it appears that opposite extremes in temperature also culminate in acid injury (**26**) and it is possible that persistent lack of vigor, irrespective of its mode of origin, may after a sufficient lapse of time result in high sap acidity (**23, 25, 26**).

The untreated loam produced more vigorous plants than the untreated, low-mineral humus though both soils were strongly acid. In this connection reference may be made to the work of ÅSLANDER (**2**) who has shown that the concentration of nutrients is apt to be more significant than the pH of the soil or culture medium. He has shown that calciphiles may develop normally in highly acid media provided the latter contain sufficiently concentrated nutrients. Acid injury occurred only when highly buffered acid cultures were diluted without change in pH. Even in such instances plants again developed normally if the hydrion concentration of the diluted cultures was reduced. ÅSLANDER concludes that many plants are highly acid tolerant if soils are rich in salts. The work of HOAGLAND (**19**) also indicates that increased availability of nutrients may cause an increase in sap alkalinity. In the present instance the higher mineral content of the acid loam may in part account for the better growth of wheat on this soil.

It will be noted (table II) that fluctuations in light intensity and soil acidity produced smaller changes in sap hydrion concentration of plants

grown on the loam soil. The greater mineral content of the loam probably results in more highly buffered tissue fluids and these in turn show smaller fluctuations in free acidity. Recent work (**50**) has definitely established the importance of mineral nutrients as buffers in plant tissue fluids. Plants adequately supplied with inorganic salts may be able by the buffer effect of these to maintain a fixed pH level and to protect themselves against acid injury. Under certain conditions plants also appear to supplement the protective effect of their buffer systems. HOAGLAND (**19, 20**) and his co-workers have shown that absorption of nitrates by plants is increased in acid media. PRIANISCHNIKOW (**40**) states that this increased absorption of nitrates from acid soils is followed by active excretion of ammonia resulting in neutralization of acidity about the roots.

The data of other investigators suggest that the effect of lime on sap acidity varies with the species and with the soil. CLEVENGER (**9**) reports that lime fertilizers applied to the same soil increased the sap hydrion concentration in oats and soybeans but not in buckwheat. HAAS (**14**) and other workers also report increased acidity in some species and diminished acidity in others following liming. Though the direction of the shift in pH appears to vary with the type of plant and the soil, HAAS and CLEVENGER have shown that lime exerts a profound effect on tissue fluid reaction in all instances. Under certain conditions, such as lack of balance among nutrients or mineral insufficiency, it seems that the effect of lime in altering the free acidity of the sap may outweigh its other functions as a nutrient.

The foregoing data show that both soil reaction and light influence sap acidity. When their effects are cumulative the change in metabolism is sufficient to cause a gradual alteration in the appearance of the plants. Neither the effect of light, nor of mineral nutrients, on sap hydrion concentration can be explained on any simple basis. The manifold importance of light as a factor influencing the carbohydrate supply, water content, mineral nutrient intake and sap acidity of plants makes it exceedingly difficult to interpret its effects. We are especially in need of additional information concerning the effects of insolation on mineral nutrition and tissue reaction. Considerable evidence stressing the importance of sap acidity as a morphogenetic factor in plant growth has already accumulated (**3, 13, 17, 18, 42**) but its accurate interpretation awaits further information concerning the factors controlling the reaction of tissue fluids.

Summary

Tissue fluids from entire wheat tops showed diurnal acid periodicity, free acidity reaching a minimum in the evening and a maximum in the early morning. Sap acidity of wheat plants on acid soils was much greater

than that of plants on the same soils after applications of calcium carbonate to correct acidity. The general level of free acidity was much lower in plants on an untreated, low mineral humus than in those on an acid loam higher in mineral matter. Symptoms of acid toxicity appeared in the tops of eight week old wheat on the untreated acid humus. Following correction of soil acidity with lime, the sap hydrion concentration of plants on the humus was reduced more than in those on the loam. The sap acidity of strongly insolated plants on limed humus soil fell below the level necessary for iron mobility, as shown by chlorosis and absence of iron in the leaves. When chlorosis had persisted for two or more weeks, leaves gradually lost their turgor and acidity rose rapidly as the moisture content diminished.

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TITRATION CURVES OF ETIOLATED AND OF GREEN WHEAT SEEDLINGS REPRODUCED WITH BUFFER MIXTURES

ANNIE MAY HURD-KARRER

(WITH SIX FIGURES)

Introduction

The titration curve for the expressed juice of etiolated wheat seedlings has a pronounced inflection above pH 8.0 (fig. 4), which does not occur in the curve for fully green seedlings (fig. 5). An intermediate type of curve, linear between pH 8.5 and 11.0, is characteristic of very young seedlings just after their emergence from the ground, when, from the standpoint of chemical composition, they are partially etiolated (fig. 6).

The characteristic inflection in the etiolated-seedling curve seemed due to some buffer present in maximum concentration in the absence of chlorophyll, and the change in the form of the curve during the period of seedling development appeared to be caused by a progressive diminution in the amount of this buffer as assimilation processes became established.

The object of the present investigation was to identify this buffer, and to determine whether the titration curves of etiolated and of green wheat seedlings could be reproduced by varying its concentration in chemical mixtures containing it in combination with other buffer compounds known to occur in plant juices.

Material

The etiolated seedlings from which juice was obtained for the representative titration curve in fig. 4 were of the variety White Odessa, grown in the darkness in pure white sand without nutrients at a temperature of 18° C. The plants were eight days old and about 8 cm. high at the time of cutting, with the first leaf just emerging from the coleoptile.

The green seedlings, from which the juice was obtained for the representative titration curve in fig. 5, were of the same variety, grown in soil under normal greenhouse conditions, at approximately the same temperature. The plants were cut eight days after their emergence from the soil. They were 8–10 cm. tall, with one fully expanded leaf.

The characteristic inflection in the etiolated-seedling curve was the same for seedlings grown in soil, pure sand, and on saturated blotting paper without nutrients. The curve for fully green seedlings never showed this inflection whether the plants were germinated in soil or in pure sand without nutrients.

The form of the green-seedling curve varies with age,—from the type suggesting partial etiolation, characteristic of emergent seedlings, to that

obtained a week or two later when the products of assimilation reach an equilibrium concentration and the form of the curve becomes that characteristic of the plant for the remainder of the growth period (**26**). The curve in fig. 5 was chosen as representative of this final stage, although some variation, apparently due largely to the decreasing water content of the plants, occurs in later stages (**26**).

Age was not a factor in the shape of the etiolated-seedling curve, which remained essentially unchanged throughout the limited period in which the plants could grow in darkness. However, seedlings younger than five days were not investigated in this connection.

While varietal differences and environmental factors such as temperature and water supply were found to affect the steepness of the titration curve, they did not change essentially the characteristic form associated with etiolation, nor that associated with normal assimilation, as long as growth was normally vigorous. If, however, the plants were not healthy, their condition was reflected in the titration curve. In the case of etiolated seedlings having weak, scraggly growth and poorly developed root systems, the characteristic inflection above pH 8.0 was less pronounced. In the case of green plants, lack of vigor was associated with greatly increased buffer capacity.

The titration values for the juice of plants of the same variety, in the same stage of development, grown under the same environmental conditions, cut at the same time, and handled identically, were remarkably constant. Each of the 45 E. M. F. measurements constituting a complete titration have frequently been reproduced with duplicate samples of juice to within a few hundredths of a pH unit. This reproducibility was particularly marked in the case of etiolated seedlings, for which growth conditions were more constant than they were for plants grown in light.

Methods

To obtain the juice samples, the plants, exclusive of the roots, were ground in a food chopper, and the juice expressed from the pulp by squeezing it through cheesecloth. Ten-cc. samples were titrated electrometrically at a temperature of 25° C. with twentieth normal acid and alkali. The method and equipment are described in an earlier paper (**23**).

The characteristic slopes and inflections of the plotted titration curves suggested the buffers to be used in the mixtures with which the curves were imitated. To determine their proper concentration, different dilutions of stock solutions of these buffers were titrated, and the concentration of each buffer in the final mixture made equal to its concentration in that solution whose titration curve most nearly coincided with the corresponding section of the juice curve, *i.e.*, the section over which the solute was

apparently the principal buffer. Adjustment of the initial reaction of each mixture to that of the juice, approximately pH 6.00, was brought about by the addition of a small amount of N/1 NaOH. Vertical displacement of the entire curve could be corrected by varying the amount of water in the mixture.

Results

The characteristic inflection of the titration curve for the etiolated-seedling juice (fig. 4) suggested the presence of an amino acid or a related compound. The location of the point of inflection near pH 8.9 indicated that the compound was probably asparagin, which has a dissociation exponent near this point ($pk_a = 8.87$ (**7**)). Asparagin has been reported to be more abundant in etiolated seedlings than in corresponding seedlings grown in light, owing to its utilization in protein synthesis in assimilating plants (**37**). WAŚNIEWSKI (**50**) has shown this relation in the case of wheat seedlings.

To determine whether asparagin was present in the juice of the etiolated wheat seedlings of the present investigation, several samples were evaporated to a thin syrup (after the heat-coagulable material had been removed by filtration) and allowed to stand for some time in a refrigerator, together with similarly prepared samples of green-seedling juice. Many asparagin crystals separated out in the etiolated-seedling preparations, whereas only potassium nitrate crystals appeared in the green-juice samples.[1] Their identity was confirmed by Mr. G. L. KEENAN, Microanalyst of the Food, Drug and Insecticide Administration, by means of the optical immersion method. It was concluded that the distinguishing inflection of the etiolated-seedling curve above pH 8.0 (fig. 4) was due to the asparagin in the juice.

The most important buffer from the standpoint of the physiology of the plant would be one which reacts with acid and alkali at and near the hydrogen-ion concentration of the tissues. The pH values obtained (**23, 24, 27**) for the juice of healthy wheat plants in the vegetative stage lie within the buffer range of the inorganic phosphates (**5, 11**). LE CLERC (**31**) and others have found phosphates to be abundant in wheat seedlings. They have been reported to be important constituents of the buffer systems of a number of plant tissues (**1, 12, 28, 33, 34, 35**). So it seemed probable that they are of similar importance in wheat.

Accordingly mixtures of asparagin and potassium phosphate were titrated in the first attempts to reproduce the titration curve of the etiolated-

[1] The absence of asparagin crystals in the green-seedling juice can not be taken to mean that asparagin was entirely absent, since crystallization of the relatively small amount undoubtedly there, might have been inhibited by interfering substances which were not present in the etiolated-seedling juice.

seedling juice. In order to minimize final adjustments of the reaction of the mixtures, a stock solution of potassium phosphate was made to have a pH value near that of the juice by adding seven parts of a tenth normal solution of the monobasic phosphate to one part of a similar solution of the dibasic phosphate. It was found after a number of preliminary experiments that the titration values for 10 cc. of a mixture consisting of three parts of this phosphate solution, 13 parts of a tenth normal solution of asparagin, three parts of water, and one drop (0.033 cc.) of N/1 NaOH to adjust the reaction, were very similar to the titration values of the juice between pH 6.0 and pH 9.5. The mixture was lacking in buffer capacity beyond these limits, however.

LEUTHARDT's data (**32,** p. 35) showing that glucose and sucrose react with alkali above pH 9.0 suggested that the addition of sugar to the mixture might give it the buffer capacity of the juice over the extreme alkaline range. After a number of preliminary trials with different concentrations, it was found that a 5 per cent. solution of glucose (1 gram to each 18 cc. of the phosphate-asparagin mixture) was required for the degree of buffer action characterizing the juice above pH 9.5. This concentration is three times that of the total sugar actually found in the juice.[2] So the sugar in the mixture must be regarded as representative of all the soluble carbohydrates of the juice and possibly of other constituents which react with alkali above pH 9.5.

The alkali-titration curve of the etiolated-seedling juice was now reproduced with phosphate, asparagin, and glucose (fig. 1, d). The buffering contributed by the potassium phosphate and by the asparagin is shown in fig. 1 (a and b) by titrations of solutions containing them in the same concentration in which they occurred in the mixture. Their combined buffer capacity is shown also with (fig. 1, d) and without (fig. 1, c) that of glucose. The buffering due to glucose, beginning near pH 9.5, annulled to just the right degree the increase in steepness of the curve above pH 10.0 due to the disappearance of buffering by asparagin.

The mixture still lacked much of the buffer capacity of the juice over the range of the acid titration (fig. 3). Organic acids in the presence of their salts have been shown to be effective buffers in plant tissues over acid reaction ranges (**20, 28, 32, 35**). The identity of these constituents in the wheat plant has not been determined. So in order to determine whether any of the most commonly occurring acids would make possible the dupli-

[2] In some analyses made by Dr. ALLAN D. DICKSON in this laboratory, 10 cc. of juice from etiolated seedlings were found to contain 0.18 gm. total sugar. The same quantity of juice from green seedlings contained 0.14 gm. The two analyses are not comparable, however, because the temperatures in the greenhouse when the green seedlings were grown were too high for normal growth.

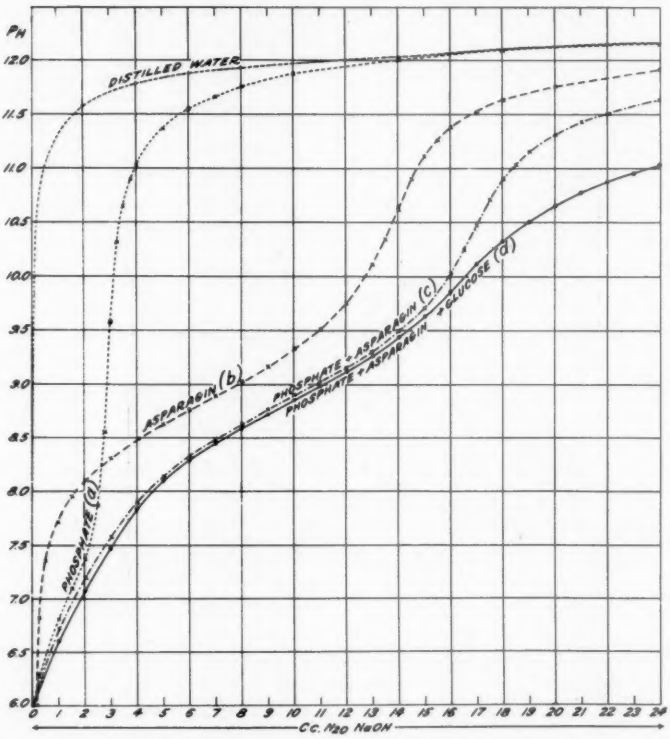

FIG. 1. Graph illustrating the synthesis of the alkali-titration curve of etiolated-seedling juice. The curves of 0.016 M potassium phosphate (a) and of 0.069 M asparagin (b), alone and in combination with each other (c), and with 5 per cent. glucose (d) show the contribution of each buffer to the form of the juice curve. The concentration of the solute in each case was approximately equal to its concentration in the whole mixture.

cation of the acid-titration curve of wheat juice, solutions containing malic, oxalic, citric, tartaric, and succinic acids were titrated for comparison of their curves with that of the juice. At pH 6.0, the approximate reaction of wheat juice, the organic acids are largely, or, in the case of some of them, entirely in the form of salts. So in order that their titrations should be comparable with those of wheat juice, the solutions of the different acids (M/10) were each brought to pH 6.0 by the addition of alkali, adjusted to equal volumes with distilled water, and then titrated similarly with acid. The addition of the necessary alkali and the subsequent volume adjustment with water reduced the original strength of each solution to 0.0645 M.

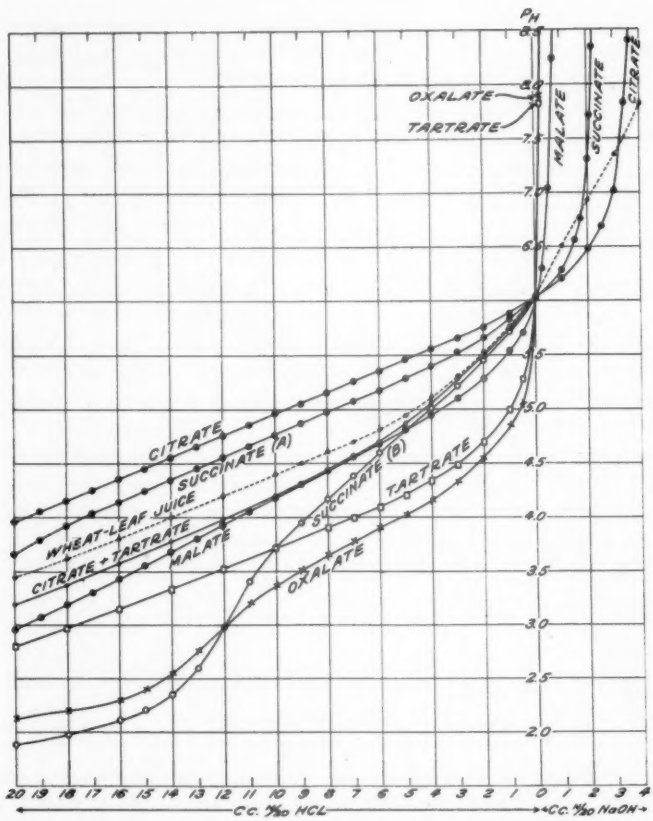

FIG. 2. Graph showing characteristic inflections and buffer zones of plant acids. M/10 solutions of each were first brought to pH 6.0, a reaction typical of wheat juice, by the addition of alkali, then titrated with acid and with alkali, respectively. A titration curve for juice of leaves of 18-weeks-old wheat plants is included for comparison. The concentration of each organic acid-salt mixture was 0.0645 M, excepting a 1: 1 dilution (B) of the succinic-acid solution (A), included to show the inflection below pH 4.0.

The titration curves of these solutions were all different, each having characteristic inflections or characteristic zones of buffer action (fig. 2). The malate solution was the only one whose buffer zone was similar to that of wheat juice below pH 6.0. The curves for the oxalate and succinate[3]

[3] A 1: 1 dilution (B) of the original succinate (A) is included in fig. 2 in order to show the inflection below pH 4.0. The corresponding dilutions of the malate, citrate, and tartrate solutions are not included because, owing to the distribution of their dissociation constants, their slopes are practically linear over the entire buffer zone under consideration.

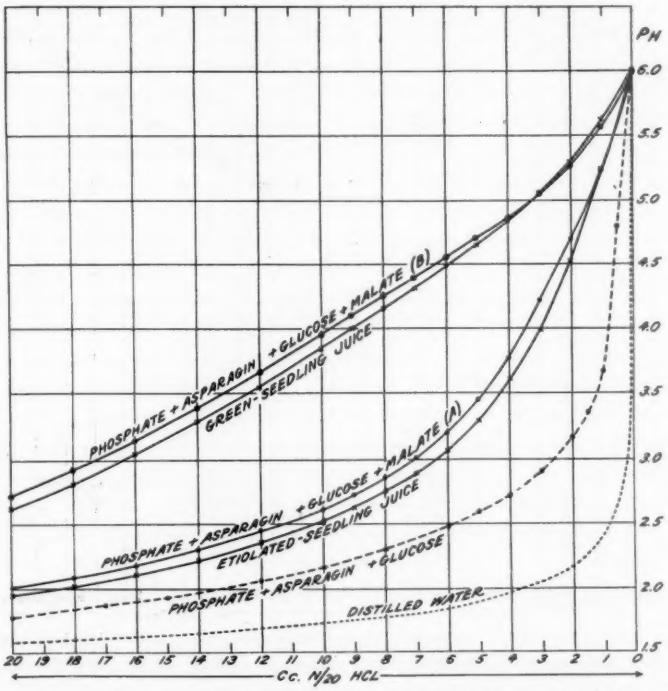

FIG. 3. Graph showing the buffering of the phosphate-asparagin-glucose mixture of fig. 1 against acid, and the effect of adding sodium malate. The concentration of malate in the solution A, imitating etiolated-seedling juice, was 0.013 M; that in the solution B, imitating green-seedling juice, was 0.045 M.

solutions had infections below pH 3.5 and 4.0, respectively, which were never present in the curve for the juice of wheat plants of any age or condition. The citrate solution had too much buffer capacity between pH 5.0 and 6.0 as compared to that below pH 5.0, and the tartrate solution had too little.

Figure 2 shows that although the curve for malates closely resembled that of wheat juice, a combination of citrate and tartrate, in the ratio of two parts of the citrate to three parts of the tartrate solution, gave a curve even more similar to that of the juice. A combination of malate, citrate, and tartrate could be made that would give similar values. Sodium malate alone, however, was chosen for inclusion in the buffer mixture.

A relatively concentrated solution was made by adding enough N/1 NaOH (19.75 cc.) to 10 cc. M/1 malic acid in the electrode vessel to bring the reaction to approximately that of the juice (near pH 6.0). By sub-

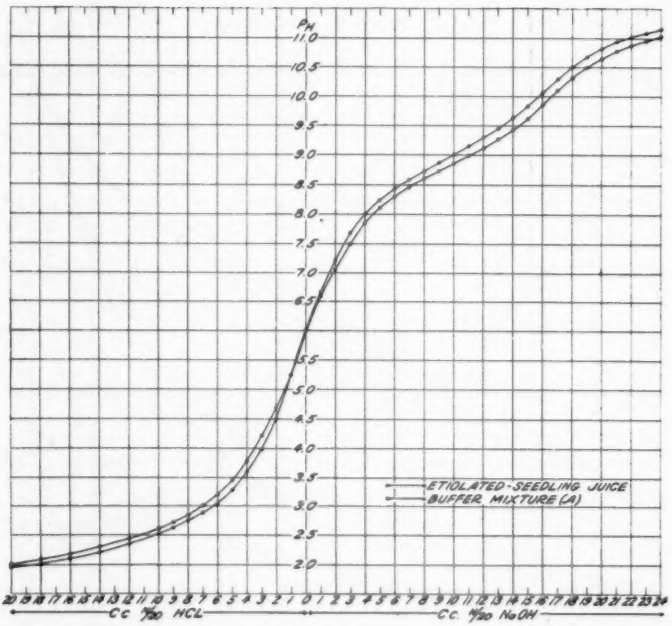

FIG. 4. Titration curve of juice of etiolated wheat seedlings, and of the buffer mixture A containing potassium phosphate, asparagin, sodium malate, and glucose.

stituting 1.5 cc. of this approximately M/3 solution for a corresponding amount of water in each 38 cc. of the phosphate-asparagin-glucose mixture, with which the alkali-titration curve of the etiolated seedlings had been reproduced, the mixture was given the desired buffering below pH 6.0 without changing appreciably its alkali-titration values.

In fig. 3 is shown the effect of this addition of malate on the acid titration values of the phosphate-asparagin-glucose mixture whose alkali-titration curve is given in fig. 1 (d). Asparagin has very little buffering power against acid, but the combined buffering of the asparagin and the phosphate in the mixture was enough to compensate for the slight deficiency in that of the malate alone between pH 5.5 and 6.0 and below pH 3.5.

The titration curve of the etiolated-seedling juice was now duplicated over its entire range with a mixture of phosphate, asparagin, glucose, and sodium malate (fig. 4). The exact composition of the mixture (A) is given in table I on p. 317.

The slight vertical displacement of the synthetic curve as compared to the juice curve—upward over the range of the acid titration and downward over that of the alkali titration,—is due to a difference in dilution.

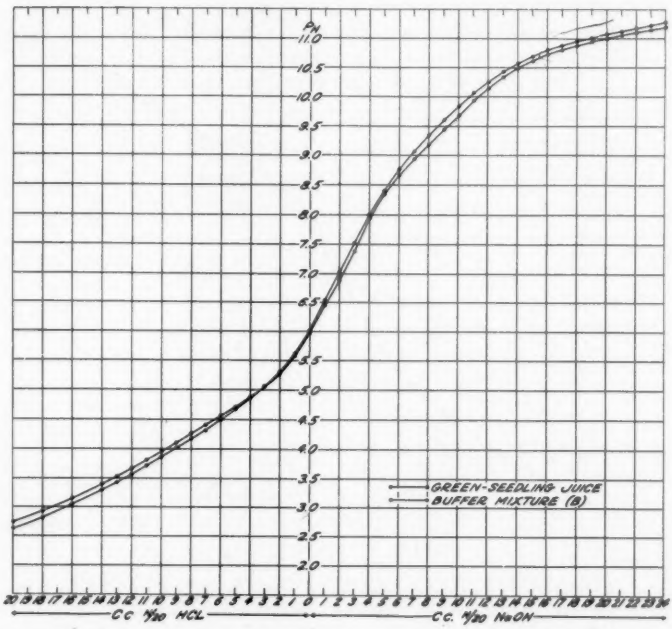

FIG. 5. Titration curve of juice of green wheat seedlings, and of the buffer mixture B containing potassium phosphate, asparagin, leucin, sodium malate, and glucose.

The constituent buffers were all slightly more concentrated in the mixture than they were in this particular sample of juice. The difference was of no greater magnitude, however, than occurs naturally between curves for different samples of juice, so the slight discrepancy was not corrected. In accordance with this difference, all subsequent mixtures were made to have a uniformly higher buffer capacity than the corresponding juices.

The titration curve for green seedlings differs from that of etiolated seedlings in not having the inflection above pH 8.0 and in having considerably increased buffer capacity over the acid range below pH 6.0 (fig. 5). These differences suggested that in order to reproduce it, the quantity of asparagin used in duplicating the etiolated-seedling curve should be decreased, and that of the sodium malate increased.

It was found that the acid-titration values of the green-seedling juice could be reproduced simply by increasing the concentration of sodium malate in the buffer mixture (fig. 3). The concentration of malate in the etiolated-seedling mixture, A, was but 0.013 M, as compared to 0.045 M in the green-seedling mixture, B. So the greater buffering of the juice of

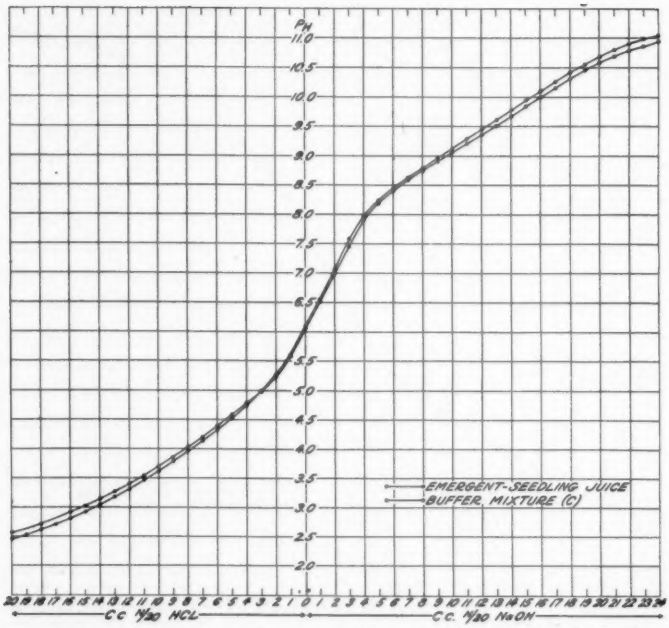

FIG. 6. Titration curve of juice of emergent wheat seedlings and of the buffer mixture C containing potassium phosphate, asparagin, leucin, sodium malate and glucose.

green seedlings against acid as compared with that of etiolated seedlings is apparently due largely to a higher concentration of organic-acid salts.

The difference between the alkali-titration curves seemed due in large measure to a lower concentration of asparagin in the green seedlings as compared to that in plants grown in darkness. However, the green-seedling curve could not be exactly reproduced simply by reducing the asparagin in the mixture, because the asparagin inflection persisted after this substance had been reduced to the minimum requisite for the buffer capacity of the juice between pH 7.5 and 9.5. The problem was then to add some substance to the mixture which would annul the tendency of the curve to increase in steepness above pH 9.5, over the region where the alkali-combining power of asparagin decreases rapidly. Doubling the amount of sugar accomplished this satisfactorily, but there was already more sugar in the mixture than was found in the juice. So leucin, which reacts with alkali at highly alkaline reactions ($pk_a = 9.75$ according to BJERRUM (7)), was used instead. The desired effect was obtained by adding M/10 leucin to the mixture in an amount equal to about half that of the minimum amount of M/10 asparagin required for the buffer capacity of

TABLE I

COMPOSITION OF BUFFER MIXTURES HAVING TITRATION VALUES SIMILAR TO THOSE OF THE JUICE OF ETIOLATED WHEAT SEEDLINGS (A), FULLY GREEN SEEDLINGS (B), AND GREEN SEEDLINGS JUST AFTER EMERGENCE FROM THE GROUND (C)

BUFFER	APPROXIMATE BUFFER RANGE *pH*	COMPOSITION OF BUFFER MIXTURE		
		A "ETIOLATED"	B "GREEN"	C "EMERGENT"
M/3 Sodium malate	2.0– 6.0	1.50 cc.	4.75 cc.	3.80 cc.
M/10 Phosphate	5.5– 8.0	6.0 cc.	6.0 cc.	6.0 cc.
M/10 Asparagin	7.5–10.0	26.0 cc.	9.0 cc.	15.0 cc.
M/10 Leucin	8.0–11.0	—	4.0 cc.	8.0 cc.
Glucose	above 9.5	2 gm.	2.2 gm.	2.2 gm.
Water	—	2.5 cc.	9.0 cc.	—
N/1 NaOH	—	0.033 cc.	0.033 cc.	0.066 cc.

the juice between pH 7.5 and 8.5—over which region neither potassium phosphate nor leucin had much buffer capacity.

The best reproduction of the green-seedling curve obtained is shown in fig. 5, and the exact composition of the buffer mixture (B) is given in table I. The imitation is not perfect, but it is as close an approximation as could be expected with so simple a mixture, lacking as it does a number of the constituents of the wheat plant which react with alkali and acid, such as cholin, betain, and such amino acids as histidin and arginin (**13, 43, 51**). However, these compounds may not be present in the juice in sufficient amounts to affect appreciably the form of the titration curve.

Table I gives also the composition of a mixture (C) with which the curve typical of normal seedlings just emerging from the ground was reproduced (fig. 6). This mixture contained less asparagin and more sodium malate than did the etiolated-seedling mixture, A, but more asparagin and less sodium malate than did the green-seedling mixture, B. As previously noted (**26**), the curve for emergent seedlings evidently represents a stage of development when the concentration of the principal buffers is intermediate between that of etiolated and of fully green seedlings.

The titration values of the three buffer mixtures, A, B, and C, are given in table II, together with the values for the corresponding juice titrations. The mixtures show in each case slightly higher buffering over both the acid and alkaline ranges than do the corresponding juice samples. Since the difference is about the same over both ranges,—the synthetic alkali curves falling below and the synthetic acid curves above the juice curves,— the titration values could be brought in still closer agreement by diluting the mixtures with a little water. However, as stated previously,

there seemed no object in exactly superimposing the curves inasmuch as different samples of juice differ even more widely in this respect.

TABLE II

TITRATION VALUES OF BUFFER MIXTURES A, B, AND C IN COMPARISON WITH CORRESPONDING VALUES FOR ETIOLATED, GREEN, AND EMERGENT WHEAT SEEDLINGS

Cc. alkali and acid added to 10 cc.	Etiolated seedling juice	Buffer mixture A	Green seedling juice	Buffer mixture B	Emergent seedling juice	Buffer mixture C
N/20 NaOH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0	6.03	5.99	6.00	5.98	6.09	6.06
1	6.65	6.59	6.53	6.44	6.55	6.51
2	7.20	7.04	7.03	6.85	7.06	6.97
3	7.68	7.48	7.52	7.35	7.58	7.45
4	7.99	7.84	8.00	7.96	7.97	7.89
5	8.23	8.10	8.40	8.37	8.23	8.20
6	8.42	8.29	8.77	8.68	8.44	8.41
7	8.58	8.45	9.08	8.94	8.63	8.59
8	8.72	8.59	9.35	9.20	8.79	8.76
9	8.87	8.72	9.62	9.44	8.96	8.92
10	9.01	8.86	9.86	9.69	9.13	9.07
11	9.15	8.99	10.08	9.94	9.29	9.21
12	9.30	9.12	10.27	10.16	9.46	9.36
13	9.46	9.27	10.45	10.35	9.61	9.51
14	9.64	9.43	10.59	10.51	9.78	9.68
15	9.84	9.62	10.72	10.64	9.95	9.83
16	10.07	9.85	10.82	10.74	10.11	9.99
17	10.29	10.11	10.91	10.83	10.27	10.16
18	10.50	10.32	10.98	10.90	10.42	10.32
19	10.69	10.51	11.04	10.97	10.56	10.47
20	10.83	10.66	11.09	11.03	10.68	10.59
21	10.94	10.78	11.15	11.08	10.80	10.69
22	11.02	10.88	11.20	11.13	10.90	10.79
23	11.10	10.96	11.25	11.17	10.98	10.86
24	11.16	11.03	11.31	11.21	11.06	10.94
N/20 HCL						
0	5.97	5.99	6.00	5.98	6.10	6.06
1	5.23	5.23	5.63	5.58	5.60	5.58
2	4.50	4.69	5.31	5.27	5.26	5.22
3	3.97	4.22	5.05	5.05	4.98	4.97
4	3.59	3.78	4.84	4.87	4.73	4.77
5	3.28	3.45	4.66	4.71	4.52	4.58
6	3.06	3.20	4.49	4.57	4.32	4.39
7	2.88	3.00	4.31	4.41	4.14	4.21
8	2.75	2.84	4.17	4.26	3.96	4.03
9	2.62	2.72	4.04	4.11	3.79	3.86
10	2.52	2.59	3.86	3.96	3.63	3.68
11	2.44		3.72	3.82	3.47	3.54
12	2.35	2.44	3.57	3.67	3.32	3.39
13	2.28		3.43	3.53	3.18	3.26
14	2.21	2.29	3.29	3.40	3.05	3.13
15	2.16		3.17		2.93	3.02
16	2.10	2.17	3.04	3.15	2.81	2.92
17	2.06		2.92		2.72	
18	2.02	2.08	2.81	2.93	2.62	2.72
19	1.97		2.70		2.53	
20	1.94	2.00	2.62	2.73	2.45	2.57

The molar concentrations of each constituent in the three mixtures are given in table III. If the assumption be made that these constituents represent the only buffers having any appreciable effect on the titration values over their respective buffer zones, their concentrations in the mixtures are approximately equal to their actual concentrations in the different juices.

TABLE III

THE MOLAR CONCENTRATIONS OF THE PRINCIPAL BUFFERS IN THE MIXTURES USED TO REPRODUCE THE TITRATION CURVES OF WHEAT-SEEDLING JUICES

BUFFER	CONDITION OF SEEDLINGS		
	ETIOLATED	PARTIALLY ETIOLATED (EMERGING FROM SOIL)	FULLY GREEN
Asparagin	0.069	0.043	0.026
Organic-acid salt represented by sodium malate	0.013	0.036	0.045[a]
Phosphate	0.016	0.017	0.017[b]

[a] FIG. 3 shows that the concentration of sodium malate required to reproduce the slope of the curve of old wheat plants over the organic-acid buffer range was still greater, *i.e.*, over 0.065 M.

[b] MARTIN (35) reports molar concentrations of phosphate of this order of magnitude in bean juice.

It may be concluded that the green-seedling juice contained about one-third as much asparagin as did that of etiolated seedlings and about three times as much of an organic-acid salt. These differences are consistent with the effects of etiolation on chemical composition as discussed in a following section.

Boiling and subsequently filtering either the etiolated- or the green-seedling juice had no appreciable effect on the distinguishing characteristics of the titration curve. So heat-coagulable proteins play little or no part in the buffer system of wheat, as is true of most other plants, apparently (**28, 33, 35, 53**). However, an exception appears in the case of potato juice, in which COHN, GROSS, and JOHNSON (**12**) found that tuberin has considerable buffer capacity over the range "acid to pH 4.5 and alkaline to pH 8.5."[4]

[4] In a preceding paper by the present writer (**26**) this statement was referred to as indicating buffer action between these limits, *i.e.*, over the range of the acid and alkali titrations from the pH value of the juice (6.8) to pH 4.5 and 8.5, respectively. From data given in another paper by Dr. COHN (**10**) it seems that while tuberin has some acid- and alkali-combining power over this range, its greatest reactivity is beyond these limits. INGOLD (28) has concluded "that tuberin, or at any rate that part coagulated by heat, has very little effect in buffering the sap." Therefore, a further statement by Dr. COHN concerning this matter may be of interest. He

The significance of titration values in plant juice studies

In a non-acid juice the organic acids are largely in the form of salts which do not react with alkali. Figure 2 shows that there can be very little titratable acid at reactions above pH 5.5 in the case of oxalic and tartaric acids, above pH 6.0 in the case of malic, above pH 6.5 in succinic, and above pH 7.0 in citric acid. WILLAMAN (**52**) has stated that titration data on plant juices have little significance since "the acids occur as salts to a considerable degree and the titration gives no idea of the absolute quantity of acids present." Only when the initial reaction of a juice is well within the range of reactivity of the organic acids and their acid salts with alkali, as in the case of such plants as *Begonia* (**40**), the acid succulents (**20**), and many fruits, does the usual titration with alkali to pH 7.0 or 8.3 include any appreciable amount of the organic-acid compounds present. HEMPEL (**20**) and LEUTHARDT (**32**) have shown the quantitative relation between the initial reaction of a plant juice and the amount of free acid which is present, the percentage at any given reaction depending on the nature of the constituent acids. HAYNES and BROWN (**19**) apply this relation in estimating the salt content of apples from the titration and pH values of their juice.

Titration of a non-acid juice may often be an accurate measure of the phosphates, whose major zone of reactivity is between pH 5.5 and 8.0. MARTIN (**33, 34**) has shown a quantitative agreement between the concentration of phosphate in the tissues of the sunflower and the buffer values of the juice between pH 5.2 and 8.0. TEAKLE's data (**48**) also show a relation between the degree of buffering of wheat juice over the phosphate range and the actual phosphate content of the juice.

If pH 8.3 be taken as the end-point, titration includes also a trace of such compounds as asparagin. In order, therefore, to determine quantitatively the concentration of any constituent in the juice by titration, consideration must be given to the pH limits of its zone of reactivity and to those of other substances whose reaction ranges might overlap this zone. Otherwise, the so-called "total acidity" measurement becomes merely a measure of total buffer capacity between specified pH limits, as is generally

says in a private communication: "There is, of course, combination of tuberin with acid and base over the physiological range, but in the case of this protein, as of most others, the largest number of reactive groups appear to have dissociation constants in the neighborhood of pH 3.3 and pH 9.7. . . . The titration curve suggests that tuberin is no exception to the general rule. Roughly, to pass from pH 4.5 to 8.5 required four parts of alkali, whereas to go from pH 8.5 to 10.5 required eight parts, and from pH 2.5 to 4.5 something over ten parts. In the case of the potato juice as a whole buffering is, of course, in part due to this protein, and in part to the phosphates and other weak acids that may be present."

recognized. It is not surprising that these determinations have been so frequently found to bear no consistent relation to the corresponding hydrogen-ion concentrations in plant juices (**16, 26**).

HEMPEL (**20**) has called attention to the fact that when aluminum salts of the organic acids are present, such as the aluminum malate in succulents, titration with alkali between pH 6.8 and 9.2 is complicated by the precipitation of the insoluble aluminum hydroxide. She thus explained the greater amount of alkali required to titrate the juice to pH 9.2 as compared to that required to bring it to pH 6.8. She has concluded that the presence of unknown substances "renders titration of plant juices to the phenolphthalein end-point uncertain or valueless."

Comparison of the points of inflection and characteristic buffer zones of the plotted titration curve of a plant juice with those of known buffer compounds may afford a means of identifying these constituents in the plant. The possibility of utilizing the characteristics of the curves of the common plant acids to identify them in mixtures is suggested by the work of AUERBACH and SMOLCZYK (**4**) and TÄUFEL and WAGNER (**47**). LEUTHARDT (**32**) has shown that the characteristics of the titration curves of apples, grapes, lemons, and tomatoes are in agreement with the nature of the constituent acids as determined by chemical analysis. He concludes, however, that when a buffer mixture contains more than one acid, it is generally impossible to identify the acids from the titration curve alone.

TAGUE (**46**) showed the value of the titration method in the identification and quantitative estimation of amino acids. Subsequently HARRIS (**18**), HIRSCH (**21**), and TÄUFEL and WAGNER (**47**) studied the titration curves of protein degradation products and pointed out their value in analyzing these compounds in mixtures. LEUTHARDT (**32**) attributed the point of inflection at pH 10.4 in the curve for *Mesembryanthemum* juice to the amide, glutamin, the presence of which he proved by chemical analysis. Similarly, asparagin has been identified in the juice of the etiolated wheat seedlings of the present investigation.

Several investigators have called attention to similarities between the form of some region of the titration curve of a plant juice and that of a known solution containing substances occurring in the juice. Thus HOAGLAND and DAVIS (**22**) pointed out the resemblance of the titration curve of *Nitella* sap between pH 5.2 and 8.0 to the corresponding region of a curve obtained with a mixture of the inorganic salts, including phosphates, that were found to occur in the sap. YOUDEN and DENNY (**53**) noted that the titration curve of apple juice was like that of a mixture containing its acid constituent, malic acid, and sodium malate. They also compared the curve for a mixture of organic acids with the curve for potato juice.

Titration values are now finding another use in quantitative estimations of buffer constituents of plant juices through comparisons of so-called buffer-index values. The slope of the tangent to the titration curve is a measure of the buffering power of the juice at any given point on the curve, and varies with the concentration of the substance being titrated. The buffer index constitutes a measure of the slope of the line connecting the two points on the titration curve which limit the region under consideration, or, the slope of the tangent at the intermediate point. The original unit, suggested by KOPPEL and SPIRO (**29**), is the amount of alkali or acid required to produce a unit change in the pH value of a solution, corrected for the amount required to produce the same change in the solvent alone. VAN SLYKE'S unit (**49**) differs in not incorporating the correction for dilution, but, as this investigator points out, between pH 3 and 11 the correction is very small and the units are essentially the same. INGOLD (**28**) has shown the effectiveness of the VAN SLYKE buffer index as a means of determining the percentage of buffering in potato juice due to phosphates, citrates, and ether-soluble substances, respectively, over each unit pH range between pH 4.0 and 7.0.

For a further discussion of the significance of the titration curve and buffer-index concepts from the standpoint of quantitative analysis of plant juices, reference should be made to LEUTHARDT'S paper (**32**).

Discussion

The question as to whether the principal buffers of the wheat plant have been identified and the buffer system approximately reproduced in proper concentration must remain problematical until complete analyses of the juice are available. However, it does not seem probable that the close agreement between the titration values of the buffer mixtures and those of the different juice samples is entirely fortuitous. It is of interest in this connection to compare the composition of these mixtures with what is known of the occurrence of their constituents in etiolated and in green wheat plants.

Asparagin.—Asparagin has frequently been reported to occur in wheat (**9, 13, 14, 41a, 43, 44, 50**). It is known to occur in greater amount in etiolated than in the green seedlings of many plants (**37**) including wheat (**50**) and other cereals (**2, 8, 41, 45**). So the fact that more asparagin was required to reproduce the curve for etiolated wheat seedlings than was used to reproduce that of green seedlings is consistent with the results of quantitative analyses. The curve for emergent seedlings, which, being partially etiolated, should contain an intermediate concentration of asparagin, was accurately reproduced on the basis of this assumption. The gradual decrease in the buffer capacity of the juice over much of the alkaline range

during the period of seedling development may therefore be assumed to reflect the utilization of asparagin in protein synthesis.

Organic acids.—The organic acids of the wheat plant have not been determined. On the basis of the form of the acid-titration curve of the juice, certain predictions may be made as to the organic-acid salts most likely to be present. Of the five commonest ones,—oxalates, succinates, tartrates, citrates, and malates,—the first two are at once eliminated by pronounced inflections below pH 4.0 (fig. 2). There is no trace of such an inflection in the curve for wheat juice. The titration curve of sodium malate is so similar to that of the juice over the acid range that it suggests that salts of this acid are the most abundant. However, fig. 2 shows that a similar curve is obtained with a combination of citrates and tartrates. It is apparent that a combination of malates, citrates, and tartrates would give practically the same curve. As LEUTHARDT (**32,** p. 30) has pointed out, it is probably impossible to identify with certainty the constituents in such a mixture by titration. However, it is obvious that salts of such acids as oxalic and succinic, having characteristically inflected curves, are not present in wheat in sufficient amounts to affect its titration values.

There is but little information available on the quantitative effects of etiolation on the acidity of any plant. Most investigators (**3, 17, 30, 38, 42**) who have worked on this problem have used titration methods which are uncertain for the present purpose, owing to the fact that phosphates and perhaps other buffers were included in the titrations. BASSALIK (**6**), however, has determined by quantitative analysis that etiolated plants of *Rumex, Oxalis* and *Begonia* contain less oxalic acid than do corresponding plants grown in the light. This finding is consistent with the fact that about three times as much sodium malate was required for the reproduction of the green-seedling curve of wheat as was used in duplicating the etiolated-seedling curve.

No data have been published, apparently, on changes in organic-acid content with seedling development. The progressive increase in the acid-combining power of the juice over the organic-acid buffer range during this period was reproduced by increasing the amount of sodium malate in the buffer mixture, suggesting the occurrence of a corresponding increase in such a buffer in the tissues as the young seedlings develop from the partially etiolated condition which characterizes them at emergence. Thus all the evidence of the titration curves is in agreement with BASSALIK'S (**6**) report and with ASTRUC'S (**3**) general conclusion that the formation of organic acids is greater in green tissues than in those without chlorophyll.

Phosphates.—ANDRÉ (**2**) reported that etiolation decreases the phosphate content of corn and lupin, but RISSMANN (**39**) found a somewhat higher concentration (as P_2O_5) in etiolated wheat seedlings than in the

corresponding tissues of the green plants. RISSMANN'S results do not at first seem consistent with the fact that etiolated wheat seedlings have slightly less buffer capacity over the phosphate range than do green ones. However, etiolated plants have a higher water content than do plants grown in light (**15, 36,** etc.). The fact that RISSMANN'S data are based on dry weights of the tissues probably explains their failure to agree with the fact that the concentration of the phosphate in the juice of etiolated seedlings seems from the titration data to be a little lower than in the juice of green seedlings.[5]

Phosphates are evidently the most important buffers in wheat inasmuch as they regulate the hydrogen-ion concentration at the reaction of the juice. The pH value during the vegetative period of plants grown in soil under normal greenhouse conditions has been found to be near 6.0 (**23, 24, 27**). This same value is characteristic of etiolated seedlings also (**25**). The ordinary reaction limits for wheat have been found to be pH 5.5 and 6.3, although occasional measurements as low as 5.3 and as high as 6.4 have been obtained. The most acid juices (below 5.7) were obtained only in late maturation stages, or in vegetative stages when the plants were unhealthy. It is not improbable that the functioning of the phosphate equilibrium in the maintenance of the proper acidity is analogous to that of the carbonate equilibrium of the blood.

Summary

1. Both the alkali- and acid-titration curves of the juice of etiolated and of green wheat seedlings have been approximately reproduced with chemical mixtures containing asparagin, phosphates, sodium malate, glucose, and, in the case of green seedlings, leucin.

2. Asparagin appears to be the substance in the juice of the etiolated seedlings which is responsible for the characteristic point of inflection of the titration curve near pH 8.9.

3. Phosphates seem to be the principal buffers between pH 6.0 and 7.5. The equilibrium between the primary and secondary phosphates may be intimately associated with the maintenance of the normal reaction of the tissues.

4. Over the range of the acid-titration of the juice, *i.e.*, below pH 6.0, the buffering seems largely due to the presence of an organic-acid con-

[5] The average water content of the tissues and specific gravity measurements of the juice of week-old wheat seedlings grown under the conditions of the present investigation were as follows:

	H_2O (PER CENT.)	SP. GR.
Etiolated seedlings	93.7	1.014
Green seedlings	90.1	1.021

stituent. A solution of sodium malate alone, or a combination of tartrate and citrate, with or without malate, gives acid-titration values similar to those of the juice. The zone of decreasing buffer capacity below pH 3.5 and pH 4.0, respectively, in the oxalate and succinate curves, giving rise to characteristic inflections which do not occur in the titration curves of wheat juice, is believed to preclude the presence of these salts in wheat juice in sufficient amount to affect the titration values.

5. Glucose supplied the requisite buffer capacity of the etiolated-seedling juice above pH 9.5. In the case of green seedlings, both leucin and glucose were used in reproducing the titration curve over this region because the requisite amount of glucose alone was so much greater than that found in the juice. The glucose was considered to be representative of all the soluble carbohydrates of the juice which react with alkali above pH 9.5.

6. The most pronounced changes in the buffer system of the wheat plant during the period of seedling development seem, from the evidence presented in this paper, to be due to a reduction in asparagin content and an increase in an organic-acid constituent as assimilation processes become established in the young plant.

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THE PHYSIOLOGY OF *CONVOLVULUS ARVENSIS* (MORNING-GLORY OR BINDWEED) IN RELATION TO ITS CONTROL BY CHEMICAL SPRAYS

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(WITH FIVE FIGURES)

I. Introduction

(a). The reaction of plants to chemical sprays, and the effectiveness of these sprays, depend upon penetration and subsequent distribution of the chemical throughout the living tissues. This paper reports some experiments relative to the factors which control these processes in the wild morning-glory (*Convolvulus arvensis*).

Early writers (**3, 5**) on morning-glory control prescribe clean cultivation, pasturing, and the use of smother crops. That these methods were unsuccessful in the hands of practical farmers was recognized in California in 1915; and in the fall of that year George P. Gray (**8**) started a series of experiments on the use of chemical sprays. A number of plant poisons were used but the application of sodium arsenite solution to the leaves of mature plants was the only treatment which he found successful. Recommendations were made (**7**) for the use of this spray in the coastal fog belt of California, but it was not extensively used by farmers. The materials were dangerous to handle and the treatment allowed the maturing of a crop of seed before the application of the spray, making eradication practically impossible.

Gray proved that the perennial roots of the morning-glory could be killed by the application of arsenic to the leaves. He realized the importance of this fact and emphasized the need for further study of the problem as the mechanism responsible for the distribution of the arsenic within the plant was not understood at that time.

Few of the workers after Gray have followed his suggestion. Publications from the United States Department of Agriculture (**17**), California (**2**), Utah (**16**), and Colorado (**14**), adhere to the older recommendations. The Colorado workers (**14**) found no evidence for translocation of arsenic in their experiments with K.M.G.[1] Better results were obtained with this product in Washington (**15**) but it was not completely satisfactory.

The present study was started in 1925 for the purpose of determining the mechanism responsible for the translocation of arsenic within the morning-glory plant. Continued attempts to control this weed by cultiva-

[1] K.M.G. is a solution of $AsCl_3$ having an acid reaction and is recommended for the control of morning-glory by the Weed Control Company of California, Berkeley.

tion methods had failed and chemicals seemed to offer the most promise. The senior author took up this work in 1926 under the late Dr. P. B. KENNEDY and published with him in 1927 (**12**) a short paper, suggesting a possible mechanism and enumerating the principal factors which tend to limit its action. JOHNSON has since suggested a somewhat different mechanism based upon an idea of GRAY'S (**8**, p. 95). The evidence presented in the present paper should aid in clarifying the problem with respect to the translocation of arsenic when it is applied to the leaves of mature plants.

(b). The killing of plants by toxic sprays depends upon two factors, namely, penetration and distribution. Movement of the spray solution from the leaf surface into the vascular strands is hindered by the cuticle and by the living cells of the mesophyll. This hindrance is most effectively overcome by the incorporation in the spray solution of acids or bases which hasten diffusion through the cuticle, and rapidly kill the underlying cells. Insect injuries often aid materially in allowing entry through the cuticle. Distribution, on the other hand, is effected through the natural conducting systems of the plant and is dependent upon the internal conditions at the time of spraying. The experimental work on this problem has led to the conclusion that water movement in the morning-glory takes place in accordance with the scheme proposed by DIXON (**6**). When the plant is actively transpiring, pressure within the xylem is reduced; the vessels, being elastic, are partially compressed, resulting in a decreased capacity, and a water deficit is set up in all of the living cells of the plant. If a solution under atmospheric pressure is released into the xylem in this condition, the sudden expansion of the vessels will cause a very rapid uptake until the vessels have attained their normal capacity. Following this a slower osmotic absorption by the living cells will continue until all deficit is satisfied. Therefore, when an extremely toxic spray is applied to the leaves and stems of a morning-glory plant and the cortical tissues rapidly killed, the sap which they contain, mixed with more or less of the toxic spray solution, will pass into the xylem vessels and be forced down toward the roots. The extent of movement will depend upon how completely the original xylem sap is removed and replaced by the descending current of solution. Instances have been observed where eosin was carried to within 20 centimeters of the growing points of roots during an exposure of one hour. In such extreme cases as these there may even have been some loss of water from the roots into the surrounding soil.

It is difficult to picture the downward movement of arsenic through the phloem, with the organic nutrients from the leaves, as suggested by GRAY (**8**) and JOHNSON (**11**), since this tissue is composed wholly of living cells in an active state. Such an active poison would rapidly kill these cells and

render the system functionless for conduction. For the same reason the latex system which may be auxiliary to the phloem in the conduction of organic nutrients (**10**) cannot be considered as effective in the transfer of arsenic.

The persistent vegetative activity of the morning-glory plant is made possible by the storage of relatively large quantities of starch in the root system. The rapidity with which these reserves may be depleted and replenished indicates the presence of a very efficient conducting mechanism. The structure of the latex vessels (**13**) indicates that at least in the storage process they may aid materially in conduction. The vessels extend from the leaves into the primary root tips and latex will exude from them wherever they are punctured. It may be forced from the vessels in short lengths of stem by means of air pressure and flows from the severed branches into water in relatively large quantities, especially if they are well illuminated. Pressure in the latex vessels seems lowest in dormant roots and highest in young actively growing tissues.

Any treatment which reduces the photosynthetic activities of the plant tends also to reduce starch reserves, but in order to be effective in control, the depletion must be complete. Elimination of top growth for one season has not always been successful, and similar treatment for three years did not eradicate an old stand in California.

Two kinds of chemical sprays have proved effective in morning-glory control, namely, arsenicals and the alkali chlorates. Only the former will be considered in this paper. The results obtained with chlorates will be left for consideration in a later publication.

II. The toxic action of arsenical sprays

A. Penetration

1. *The reaction of the spray solution.*—In order that the above pictured mechanism may function effectively in the distribution of arsenic throughout the plant, the spray solution must perform two functions. First, it must penetrate the cuticle and rapidly kill the cortical tissues, allowing the arsenic to enter the vascular strands; and second, it must supply arsenic in such a concentration that, even after considerable dilution, a lethal dose is delivered to all living tissues of the root. Acid (**1, 4**) and alkali are among the cheapest and most effective materials for killing plant tissues. The results of a comparative test of several common agents are given in figures 1, 2, 3, and 4. In figs. 1 and 2 injury is plotted against exposure time, in figs 3 and 4 injury is plotted against time, the exposure time being noted on the curves. These records are of visual injury occurring to young morning-glory shoots allowed to hang in the solutions for the given "ex-

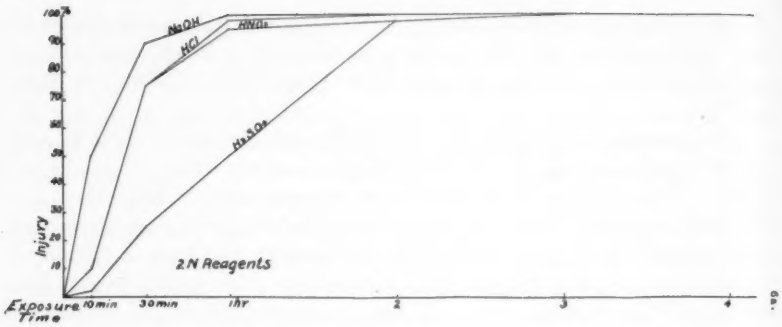

FIG. 1. Injury by reagents, 2N strength, with exposure time.

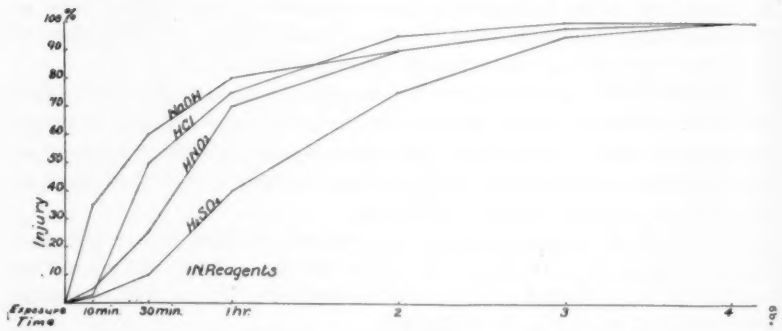

FIG. 2. Injury by reagents, 1N strength, with exposure time.

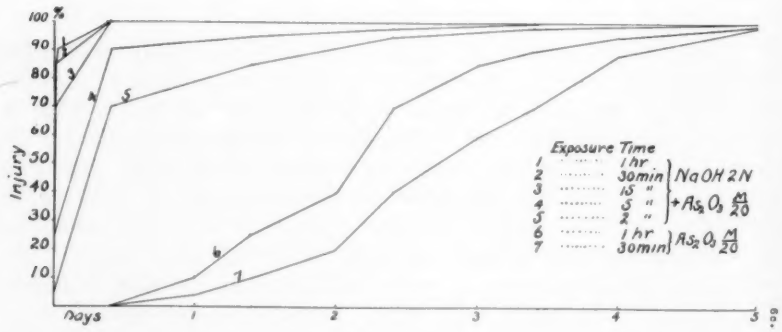

FIG. 3. Injury with alkali and arsenious oxide, with time.

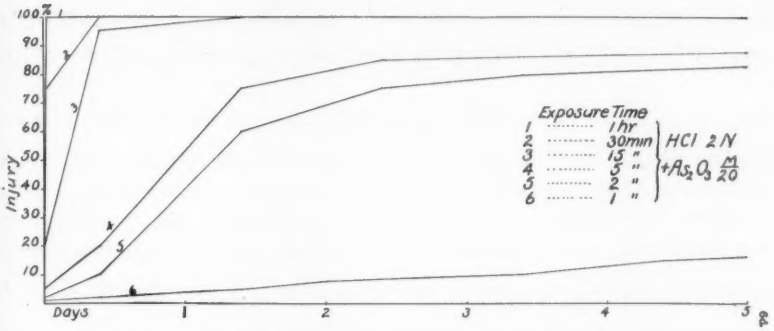

FIG. 4. Injury with acid and arsenious oxide, with time.

posure time'' and then removed, washed in tap water and set up in flasks of tap water to allow the injury to develop. The cut ends were protected from the solutions by paraffin coatings and kept above them during the exposure. Under these conditions NaOH proved the most toxic, HCl and HNO_3 were about the same, and H_2SO_4 was less toxic. The addition of $\frac{M}{20}$ As_2O_3 did not affect the initial toxicity of these agents and in neutral solution it acted very slowly upon the tissues. The acid solutions caused a light brown discoloration and the basic solutions a darkening of the green color.

To determine the pH range in which acids and bases are effective, morning-glory shoots were set up in flasks and allowed to absorb through their cut ends arsenic solutions in concentrations of $\frac{M}{20}$, $\frac{M}{80}$, $\frac{M}{320}$, $\frac{M}{1280}$, and $\frac{M}{5120}$, each concentration including solutions having pH values of 1, 3, 5, 7, 9, and 11. The solutions were buffered with phosphate and the pH values adjusted daily. The results indicated that solutions lying between pH 3 and pH 9 do not cause immediate injury due to their pH value. Injury due to arsenic was evident after 20 hours and in the three higher concentrations it was complete within 48 hours. It was necessary for the shoots in the two weaker arsenic concentrations to accumulate arsenic in the leaves before injury became apparent. A similar test using K.M.G., $AsCl_3$, Na_2HAsO_3, and As_2O_3 in comparable concentrations showed that the same conclusions applied to these chemicals. The progress of acid injury in the stems standing in the pH 1 solutions was slow compared with the rise of arsenic or eosin solutions. This indicates that the plant buffers are able to neutralize hydrogen-ion while the arsenic continues to rise in the stems.

Plants growing in the greenhouse in soil tubes were sprayed with solutions of 1N HCl, 1N NaOH, $\frac{M}{20}$ As_2O_3 in neutral solution and with 1N HCl and NaOH solutions containing $\frac{M}{20}$ As_2O_3. The acid and alkaline solutions rapidly killed those tissues with which they came in contact, the neutral solution of As_2O_3 slowly killed the tissues to which it was applied, but the solutions containing acid or alkali plus arsenic killed the root tissues to some depth below the place of application. As_2O_3 has a maximum concentration of about $\frac{M}{20}$ in neutral saturated solution. The fact that this is approximately the optimum concentration for arsenic in a spray solution indicates that more arsenic would be of no avail after the plant buffers had neutralized the acid or alkali of the spray.

Comparison of NaOH, HCl, and H_2SO_4 in arsenic solutions applied in field trials at Davis has shown that the acid solutions are the most effective. When the concentration of alkali becomes great enough to cause rapid killing, its softening action upon the plant tissues causes them to collapse and the absorption of the arsenic is hindered.

B. Distribution

1. *Water relations of the plant as affecting the movement of arsenic.*— In studying the movement of arsenic through xylem tissues it was necessary to ascertain whether or not adsorption phenomena or other chemical reactions with the surrounding walls or cells would interfere with its passage through the narrow channels. Many basic dyes are adsorbed to lignified walls, certain inorganic acids react with buffers in the tissues, and oxidizing agents may be reduced in their passage through xylem vessels. A set of preliminary tests using acid, basic and neutral solutions of arsenic in conjunction with eosin and other dyes showed that arsenic either in an anion or as a cation moved readily through morning-glory stems and roots without being rapidly withdrawn from the solution. In table I the time required for arsenic injury to become visible after reaching the tissues is shown. In this experiment morning-glory shoots were set up in arsenic solutions of the concentration given in the first column of table I. Comparable sets in eosin solution were used to indicate the rate of ascent of the transpiration current. The chief interest in this data lies in the close correlation between transpiration and the occurrence of arsenic injury. Since it takes about twenty hours for enough cells to die and become sufficiently discolored to produce visible signs of injury under these conditions, the increase in transpiration due to the breeze from an electric fan had no

appreciable effect on the shoots in the higher concentrations; but on those in the lower ones which required accumulation in the leaves to reach a lethal concentration, a marked effect may be noted.

TABLE I

EFFECT OF TRANSPIRATION UPON THE MOVEMENT OF ARSENIC INTO MORNING-GLORY SHOOTS

As_2O_3 Conc.	Light breeze		Open room		Moist chamber	
	Eosin[1]	Arsenic[2]	Eosin[1]	Arsenic[2]	Eosin[1]	Arsenic[2]
$\frac{M}{20}$	½ hr.	24 hrs.	1 hr.	24 hrs.	24 hrs.	48 hrs.
$\frac{M}{80}$	½ hr.	24 hrs.	1 hr.	24 hrs.	24 hrs.	72 hrs.
$\frac{M}{320}$	½ hr.	34 hrs.*	1 hr.	34 hrs.	24 hrs.	72 hrs.*
$\frac{M}{1280}$	½ hr.	48 hrs.	1 hr.	72 hrs.	24 hrs.	108 hrs.
$\frac{M}{5120}$	½ hr.	72 hrs.	1 hr.	168 hrs.	24 hrs.	No injury

[1] Time for complete filling of the vascular system.

[2] Time for appearance of arsenic injury.

* Only 4 shoots were subjected to each treatment and the large experimental error renders these data only roughly quantitative.

The period of twenty to twenty-four hours required for arsenic injury to become visible may have been required for accumulation to a lethal concentration, or the injury may simply have been due to a slow reaction. Eosin will kill the leaves in about four hours so this time is not all required for diffusion. Table II gives data on an experiment designed to clarify this point. Shoots were gathered, allowed to stand in tap water in the laboratory for two hours, and the cut ends placed in a $\frac{M}{20}$ As_2O_3 solution. At definite intervals sets of four shoots were removed, washed under the tap, and set up in flasks containing water. The first row of table II gives the time intervals during which the sets were allowed to absorb arsenic solution. The percentage of injury was estimated visually. Sufficient arsenic to completely kill the tissue was being absorbed within one hour, but injury was apparent only after about twenty hours. The injury to the shoots which had only a short time to absorb was different from the usual type, appearing first in the young tender stems just below the tips, spreading down the stem and out the petioles and mid-ribs, and affecting the mesophyll to the least extent. This is typical of injury by very dilute solutions and indicates that the vascular tissues are more susceptible than mesophyll.

This agrees with the fact that the lethal concentration in leaves is higher than in roots where the parenchyma of the vascular system is the first tissue to show injury.

TABLE II

ARSENIC INJURY TO MORNING-GLORY SHOOTS WHICH WERE ALLOWED TO ABSORB $\frac{M}{20}$ As_2O_3 SOLUTION FOR VARYING PERIODS OF TIME

ABSORPTION TIME	ARSENIC INJURY			TISSUES CONTAINING EOSIN*
	AFTER 24 HOURS	AFTER 29 HOURS	AFTER 42 HOURS	
hours	*per cent.*	*per cent.*	*per cent.*	*per cent.*
0.25	0	0	25	25
0.50	0	25	100	50
0.75	50	50	100	75
1.0	75	100	100	100
1.5	75	100	100	100
2.0	100	100	100	100
2.5	75	75	100	100
3.0	75	100	100	100
4.0	75	100	100	100
5.0	100	100	100	100
6.0	100	100	100	100
8.0	100	100	100	100
17.0	100	100	100	100
24.0	100	100	100	100

* Estimated visually: after two hours there was a visible accumulation of eosin in the tissues; after four hours the tissues were injured; and after five hours death occurred.

Since eosin and arsenic solutions move unhindered through xylem tissues, if stems are cut under these solutions, the rate of uptake should be an index of the amount of water deficit existing in the plants at the time the stems are cut. In table III rates of eosin uptake by stems are recorded for plants which were growing under different conditions of soil moisture, humidity, temperature, and illumination. The extent of movement was determined by removing the cortical tissues and observing the stained xylem.

Rates measured in roots proved even greater, the xylem vessels being larger. The effect of air in the vessels was shown by a series of tests made by cutting the stems in air and immediately immersing them in the eosin solution. The stems were on the same plants as those which gave the rate of 2.46 inches per second when cut under the solution and twenty-six of them gave an average rate of 0.9 inches per second. The larger vessels

TABLE III

RATES OF FLOW OF EOSIN SOLUTIONS INTO MORNING-GLORY STEMS CUT UNDER SOLUTION. (MOVEMENT RECORDED FROM CUT END TOWARD CROWN OF PLANT)

PLACE	PART OF PLANT AND GROWING CONDITIONS	TIME	TEMPERATURE	HUMIDITY	EXPOSURE	NUMBER OF TESTS	RATE, INCHES PER SECOND
			° *F.*	*per cent.*	*seconds*		
At Berkeley:							
Laboratory	Potted plant from greenhouse		70		30	3	0.18
Laboratory	Plant in water culture solution		70		300	5	0.00
Greenhouse	Potted plant in greenhouse; soil dry		90		10	4	0.82
Greenhouse	Potted plant in greenhouse; soil dry		90		10	9	0.75
Greenhouse	Plant in water culture		90		10	4	0.70
Greenhouse	Plant in water culture; petiole cut and exposed		90		30	1	0.11
Field	Plant in moist soil; petiole cut and exposed		70		30	1	0.40
At Davis:							
Field	Mature plants in dry soil	2: 30–3: 00 P. M.	83	26	5	30	2.42
Field	Mature plants in dry soil	5: 15–5: 30 P. M.	80	40	5	20	2.46
Field	Mature plants in dry soil	7: 30–8: 00 P. M.	69	56	5	20	1.86
Field	Mature plants in dry soil	5: 30–6: 00 A. M.	48	70	5	20	1.26
Field	Plants in blossom, in dry soil	4: 15–4: 30 P. M.	81	38	5	16	1.53
Field*	Mature plants in wet soil	4: 45–5: 00 P. M.	80	40	5	10	1.64
Field*	Mature plants in wet soil	7: 00–7: 30 P. M.	71	55	5	25	0.98
Field*	Mature plants in wet soil	6: 00–6: 15 A. M.	76	53	5	20	0.45

* These plants were growing in a plot which had been irrigated the day before these tests were made.

were filled with air and this movement was taking place through the smaller ones which, due to the fact that their menisci were able to withstand the pressure difference which existed at the time the cut was made, had not lost their liquid contents.

This rapid uptake of a solution under the conditions of the experiment shows that a sub-atmospheric pressure exists in the xylem of morning-glory plants which are transpiring. That the water deficit thus developed is capable of causing a thorough distribution of a solution through the roots is indicated by the fact that when the tops of plants were cut off at the ground level under eosin the roots were filled to a depth of seven feet within one hour; and if arsenic solutions were used the roots were killed to a depth of seven feet or more (**12**).

The effect of soil moisture upon the depth of penetration of arsenic was shown by an experiment using plants growing in soil tubes in the greenhouse. Three series were used, the first having been watered daily, the second having been left for five days without being watered, and the third having the tubes containing the roots and surrounding soil submerged in water for one hour before the spray was applied. Four solutions were used as shown in table IV and the extent of injury is given, the roots having been removed three weeks after the spray was applied.

TABLE IV

DEPTH OF INJURY TO SPRAYED PLANTS

SOLUTION	SOIL NORMAL	SOIL DRY	SOIL SATURATED
HCl 1.6 N	Stems killed to crown	Stem killed to crown	Stems killed to crown
As_2O_3 $\frac{M}{20}$ in HCl 1.6 N	Stem killed. Root killed 4″ below crown	Stems killed. Roots killed 12″ below crown	Stems killed to crown
As_2O_3 $\frac{M}{20}$ in NaOH 0.5 N	Stems killed. Roots killed 2″ below crown	Stems killed. Roots killed 6″ below crown	Stems killed to crown
As_2O_3 $\frac{M}{20}$ pH 7.	Stems killed to crown	Stems killed to crown	Stems killed to crown

Interpretation of a large amount of field data on spray tests leads to the same conclusion as this experiment, namely, that the dryer the soil, other conditions being equal, the deeper will the arsenic penetrate.

2. *The effect of exposure time upon the extent of injury.*—If the spray solution does not have time enough to penetrate to the vascular tissues before it becomes dried on the leaves it cannot be carried to the remote parts

of the plant. GRAY experienced this difficulty and could only obtain results (17) in the fog belt. Spraying in the afternoon when transpiration is at its height should favor deep penetration; and repeated spraying with water, following the initial application of arsenic, should avoid the drying difficulty. The two plants shown in figure 5 were sprayed with an acid solution of arsenic, plant A being allowed to dry and plant B being kept wet for two hours following the initial application. These plants had young foliage and were growing in a wet field so that distribution of the arsenic was not thorough in either of them, but the effect of a long exposure time is apparent in plant B despite the lower top : root ratio in this plant. The manufacturers of K.M.G. recommended that applications be made after sundown and during the night. Two plots of heavily infested land were used to test the effects of evaporation. One was sprayed in the afternoon, and followed by applications of water which kept the plants wet for one hour after the initial treatment; the other plot was sprayed after dark. Examination of the plants two weeks later showed no apparent difference in the effectiveness of the treatments. Evidently the night spraying did nothing except extend the exposure time. The advantage gained by the use of sulphuric acid in arsenic sprays, while partially due to the rapid killing and hardening of the plant tissue may also depend upon its hygroscopic nature.

C. Arsenic content of sprayed plants

Quantitative analyses were carried out on twenty-seven different morning-glory plants which had been sprayed with arsenic solutions. The plants were dug about ten days after spraying, the roots washed under the tap, and, after evaporation of surplus water, samples were taken and dried at 80°. These plants were growing in Berkeley and their roots branched at a depth of two feet or less and did not penetrate deeper than four feet. Table V shows the distribution of arsenic in the roots, being expressed as percentage As_2O_3 in the dry weight of a sample.

Twenty samples of stems and leaves gave an average percentage composition of 0.2011 per cent. As_2O_3. The concentration below which no injury had been noted in the fresh sample was 0.0003 per cent. in the roots and 0.02 per cent. As_2O_3 in the tops. This latter concentration was not determined on sprayed plants but on tops which had absorbed arsenic through branches cut under arsenic solution.

III. Discussion

The difficulties which caused GRAY (7) to limit the use of his sprays to the fog belt in his recommendations can be understood in the light of the

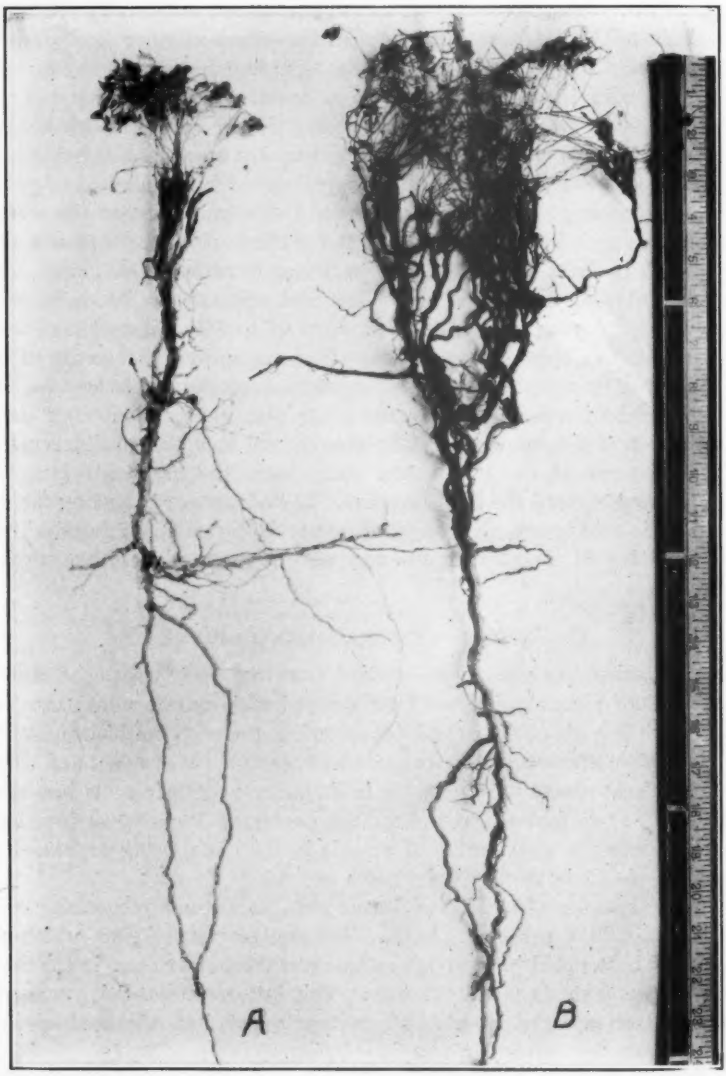

FIG. 5. Two plants sprayed with a solution having 16 parts of water to one of K.M.G. A. Plant allowed to dry after spraying. As_2O_3 content 0.041 mg. B. Plant kept wet for two hours by repeated spraying with water. As_2O_3 content 0.705 mg. Injury present to a depth of 12 inches.

TABLE V

ARSENIC DISTRIBUTION IN MORNING-GLORY ROOTS

DEPTH IN GROUND	As_2O_3 CONTENT, DRY WEIGHT BASIS
inches	*per cent.*
2	0.00508
2 to 4	0.00448
4 to 6	0.00422
6 to 8	0.00302
8 to 10	0.00262
10 to 12	0.00231
12 to 15	0.00112
15 to 18	0.00044
18 to 24	0.00030
24 to 30	0.00001

foregoing experiments. Using an alkaline solution, if he increased the alkalinity to get more rapid penetration, the gelatinizing action of the free base caused clogging of the conducting channels. If he increased the arsenic he was soon limited by its solubility in the neighborhood of neutrality. The advantage of maturity of the plants was probably more closely related to water deficit than to downward transport of organic nutrients.

The benefits of an acid solution have been pointed out. The most effective arsenicals thus far tried have had an acid reaction. The use of arsenic as a toxic agent, however, is attended by certain inherent difficulties. Besides its low solubility at the pH of plant sap it requires the use of a killing agent to effect penetration of the cortical tissues. This agent rapidly destroys the mechanism responsible for the water deficit necessary for the transport of the arsenic. Once the spray is applied and the reaction of the plant has taken place, if the treatment has not been successful nothing more can be done during that season. Plants injured to a depth of a foot or more but not completely killed seem more resistant to further treatments the following season than do untreated plants.

The poisonous nature of arsenic necessitates its transport through nonliving channels in lethal concentrations. This practically limits its movement within the plant to the xylem and prescribes a high water deficit and particularly favorable conditions for application of the spray. These conditions are seldom met in actual practice.

The latest developments in spraying technique take advantage of the water naturally occurring in the plant. Application of spray to the crown and stems allows early penetration in these regions. Subsequent spraying of the leaves releases a relatively large volume of sap into the xylem vessels resulting in a deep injection of the solution which has already

entered through the stems and which is consequently at the forward rather than at the rear end of the moving water columns. A thorough wetting of the plants is important also, for as soon as the available liquid has moved down into the roots all action ceases and the effectiveness of the treatment is fixed.

From a practical standpoint the use of arsenic as a herbicide has several advantages. It is cheap and easily obtained, it may be readily dissolved by strong reagents and later diluted for field use, and it has proved successful at least in some instances. The fact that its transport is limited to the xylem, however, almost precludes the possibility that its use can ever result in complete eradication of morning glory, for the conditions required for its thorough distribution do not occur until after the maturity of the first seeds. Possibly it can be used in some stages of a control program.

IV. Summary

1. The persistent vegetative activity of the morning-glory is related to the large storage of starch in its roots. Early control methods, based on an attempt to deplete this starch reserve, have not proven practical.

2. Killing of plants by arsenicals depends upon two factors: (a) penetration, and (b) distribution.

3. Acid solutions are more effective than basic ones in penetrating cortical tissues in the morning-glory.

4. Solutions lying between pH 3 and pH 9 do not cause rapid injury to tissues, the direct effect of their pH value being negligible.

5. Plant buffers are active in neutralizing acid and alkaline sprays.

6. Arsenic trioxide is soluble to a concentration of about one-twentieth molal at the pH of plant sap.

7. Arsenic moves freely through the xylem of morning-glory plants. Cut shoots may take up enough $\frac{M}{20}$ As_2O_3 solution in one hour to be completely killed. Injury appears only after a lapse of about 20 hours. Eosin may enter cut stems and move toward the roots at a rate as high as 2.46 inches per second for five seconds. The rate decreases as water deficit becomes satisfied.

8. Water deficit, as indicated by the uptake of eosin solution, is correlated with soil moisture and transpiration.

9. Prolonged exposure to the spray solution as by the condensation of dew or addition of water to the leaves greatly increases the depth of penetration of the arsenic.

10. The hygroscopic properties of sulphuric acid and its hardening effect upon tissues make it an effective ingredient of the spray solution.

11. Maturity is an index of the proper time for spraying only as it is accompanied by water deficit. An examination of the pressure conditions in the xylem is a much more accurate test.

12. The lethal concentrations of arsenic were 0.02 per cent. of the dry weight in tops and 0.0003 per cent. in roots.

13. Arsenic has been used with some success in the control of morning-glory but complete eradication is seldom accomplished by its use.

Acknowledgments

Most of the experimental work described in this paper was done by the senior author under the supervision of the late Dr. P. B. KENNEDY, and the original manuscript was prepared in conjunction with him. The present paper, however, has been entirely revised and rewritten since his death and so, while acknowledging his valuable guidance and aid throughout the course of the work, I assume personal responsibility for all of the material presented. I wish to express my gratitude to Dr. J. P. BENNETT for constant assistance in an advisory way as well as in the preparation of the manuscript, and to Professors B. A. MADSON and W. W. ROBBINS who have helped on several occasions.—A. S. C.

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DETERMINATION OF THE PERCENTAGE OF SUCROSE IN SUGAR BEETS FOR RESEARCH PURPOSES

DEAN A. PACK[1]

(WITH TWO FIGURES)

Introduction

Recently an article was published by REED (9), the purpose of which was: "To point out a source of error in the methods now used in sugar-beet testing and to present a method in which this particular error will not be possible."

The cold water digestion method is the one with which this author finds fault. He reports evidence of its inaccuracy and explains that, since all the cells containing sucrose are not broken when pulp samples are taken, complete extraction cannot be expected with the use of cold water. He proposes a method in which the principal innovation is that the sample, either as pulp or as chunks taken with a knife, with the necessary lead acetate added, is steamed at 15 pounds pressure for 15 minutes. The purpose of the autoclaving is to render all of the cells in the sample permeable to sucrose. Working with this autoclave method he reports that all the samples taken from an individual beet gave the same reading.

The cold water process is of such general use, both in research work and commercial practice, that REED's criticisms and recommendations should be given careful consideration.

This article points out some serious sources of error in the REED method, again demonstrates the reliability of the cold water digestion method, and offers a suggestion as to why, contrary to a tenet held in some quarters, the cold water extraction is effective.

While no attempt will be made to explain the results reported by REED, a variation of 2.5 per cent. sugar between samples from the same beet, which he noted in the cold water digestion method, could be explained by the variation in the percentage of sucrose in the different regions of the same beet. Other investigators have found wide differences in the percentage of sucrose in various regions of the beet. FLODERER and HERKE (3), who worked extensively on this question, reported average percentages of sucrose from different regions of the sugar beet, as follows: 11.51 for crown, 14.76 for shoulder, 17.59 for region of highest sugar, and from 14.40 to 16.01 for lower part of beet. They found in one test that the composited samples of two adjacent pieces from the shoulder region of fifty beets gave respectively

[1] Formerly Associate Agronomist in Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture.

14.76 and 17.29 per cent. sucrose. As these results are averages from many beets, one finds still greater difference in dealing with such samples from individual beets. It is impossible to reconcile these known variations between samples, with the statement by REED (p. 368) in which it is reported that twelve samples from one beet each gave a ten per cent. sugar content value.

REED is undoubtedly correct in his observation that ground beet pulp frequently contains unmacerated bits of tissue consisting of aggregates of unbroken cells. Obviously the common practice of eliminating, before weighing the pulp samples, such pieces of tissue as are large enough to be detected macroscopically amounts to a tacit recognition of the fact that the pulp as used may occasionally contain unmacerated bits of tissue and that fineness of pulp is desirable. It is indeed improbable that any rasp has been or will be designed which will break every cell in the pulp ground. If, in addition to granting this contention, one accepts the opinion expressed by REED that, "any cells which are not broken will not only retain their sugar but will also take up water," errors would reasonably be expected, as he maintains, in the cold water digestion method. The following experiments afford data bearing on the matter.

Experimental

MATERIALS AND METHODS

The sugar-beet sampling machine, which was the pulping device used in this investigation, has been previously described by the writer (**6**). It will hereafter be referred to as "the machine." In 1924, S. F. SHERWOOD, biochemist, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, analyzed by both the hot and cold water digestion methods sugar-beet pulp produced by this machine. He concluded that the pulp produced by this machine was fine enough for cold water digestion. The writer has since confirmed his results by checking the cold water digestion method against the hot alcohol extraction method (**1**).

The apparatus used in the analyses here reported is calibrated for one-half the normal weight of beet pulp, or 13 instead of 26 grams, and the smaller amount of pulp was consequently used for the samples. The polariscope readings are therefore one-half the actual percentage of sucrose. The samples of pulp except for experiment no. 5 (in which the weights are recorded) and those of sucrose were all weighed to an accuracy of one milligram on an analytical balance. With one exception, which will be noted, 2.6 gram samples of pure sucrose were used, so that the polariscope readings would be of about the same range as those with beet pulp samples.

The clearing of the pulp samples was accomplished by the use of a small amount of basic lead acetate solution (about 2.5 cc. of 1.25 sp. gr., or 30° Baumé, to 13 grams of sugar-beet pulp). In cases where digestion and clearing were effected at the same time, 88.5 cc. of dilute, sp. gr. 1.0073 basic lead acetate were used for each 13-gram sample. It should be noted that the amount of clearing solution used in these experiments was in accordance with standard procedure (1) rather than the concentrated solution (usually used as a stock solution) which REED advises. The clearing solution was measured by a standardized overflow burette, or weighed, and the weights corrected for the density of the solution. In all cases the volumes of the solutions were adjusted before polarization. The polariscope readings were made at 20° C. or the temperature correction applied. The readings for single samples, here recorded to one one-hundredth of a per cent., are the average of three or more separate readings on the same sample. Whenever possible, the help of a second person was obtained to check all readings.

The sugar beets used were fresh and in ideal condition for comparable sampling. Two lots of sucrose were used in the tests: (1) Domino cane sugar, refined by the American Sugar Refining Company, and (2) Saccharose "Difco" Standardized, guaranteed of perfect rotation, containing minimum moisture and ash; chlorides, sulphates, and heavy metals absent. This product is produced by the Digestive Ferments Company. The basic and neutral lead acetates used were of C. P. grade. Distilled water was used for the dilutions.

A twenty-five quart aluminum autoclave was used for these experiments. This autoclave is provided with a standard steam gauge reading in pounds from 1 to 30. It was heated on a coal stove and carefully watched by one person to control the pressure as desired. From 10 to 15 minutes heating was required to bring the pressure to 15 pounds, and 7 to 10 minutes to completely reduce this pressure.

PROCEDURE AND RESULTS

EXPERIMENT NO. 1.—Three samples of pulp were cut, with the machine mentioned, from one sugar beet. These samples were digested in cold water, cleared with basic lead acetate and polarized. The readings[2] were 10.40, 10.43, and 10.71, respectively, or an average of 10.51.

From the same beet, three comparable samples were cut with a knife. These samples were sliced into pieces one-sixteenth of an inch thick and autoclaved with basic lead acetate at 15 pounds pressure for 15 minutes. The readings were 9.24, 9.18, and 9.29, respectively, or an average of 9.24.

[2] To get the percentage of sucrose these readings should be multiplied by 2.

This indicates that treatment of one-sixteenth inch slices of sugar-beet tissue in basic lead acetate with steam at 15 pounds pressure for 15 minutes resulted in lowering the average polariscope reading 1.27, which is equivalent to 2.54 per cent. sucrose.

EXPERIMENT NO. 2.—Two samples were cut with the machine from a uniform beet at the positions indicated by sectors 1-*d* and 2-*d* in figure 1. After cold water digestion these samples read 10.07 and 9.85, or an average of 9.96.

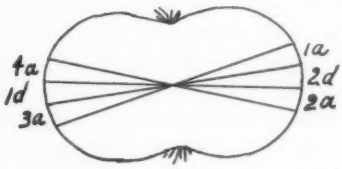

FIG. 1. Cross-section of sampled beet in experiment no. 2 showing position of samples.

Four other samples were cut with a knife from the same beet at positions indicated in the diagram (fig. 1) as 1*a*, 2*a*, 3*a*, and 4*a*. These four samples were each cut into slices one-sixteenth inch in thickness, then treated with basic lead acetate and autoclaved according to Reed's recommendation. The readings from these were 8.50, 8.75, 8.92, and 8.80, respectively, or an average of 8.74.

The REED method in this case gave an average reading showing 2.5 per cent. less sucrose in the samples than was determined by the standard cold water digestion method.

EXPERIMENT NO. 3.—Two composite samples of pulp were cut with the machine from eight beets. After cold water digestion and the usual clearing the extracts read 9.35 and 9.91, or an average of 9.63.

From the same eight beets two comparable composite samples were cut with a knife from the tissue adjoining the machine cuts. After steaming under pressure, as recommended, with basic lead acetate the extracts read 8.35 and 8.90, or an average of 8.63.

Here again the REED method resulted in a very serious error in the sucrose determination, a decrease of 2 per cent. from that determined by the cold water process.

EXPERIMENT NO. 4.—Two composite samples were cut with the machine from five uniform beets. The pulp samples were retained for analysis and the five beets were placed in a closed can and taken to the laboratory of Dr. M. D. THOMAS at the Experiment Station of the American Smelting and Refining Company. Dr. THOMAS then analyzed the beets by the REED method. Following a suggestion from the writer's work on beet tissue

pieces of different sizes, Dr. THOMAS used some samples consisting of thin slices and others of thick slices. The extracts from the thin slices averaged 18.40 per cent. sucrose and those from the thick slices 15.87 per cent. sucrose. The pulp samples, which the writer analyzed, gave an average reading of 10.12, or 20.24 per cent. sucrose.

Again the REED method indicated a distinctly lower percentage of sugar than was shown by the cold water method.

EXPERIMENT NO. 5.—Following a test in which the writer found with the autoclave method that the polariscope readings decreased with an increase in the size of the pieces of beet tissue in the sample, an experiment was planned to clarify this question.

Eight similar samples were cut with a knife from a uniform beet (see fig. 2). Precautions were taken in the sampling with regard to the known facts concerning the distribution of the sugar in sugar-beet roots (**3, 7, 8, 10,** and **12**).

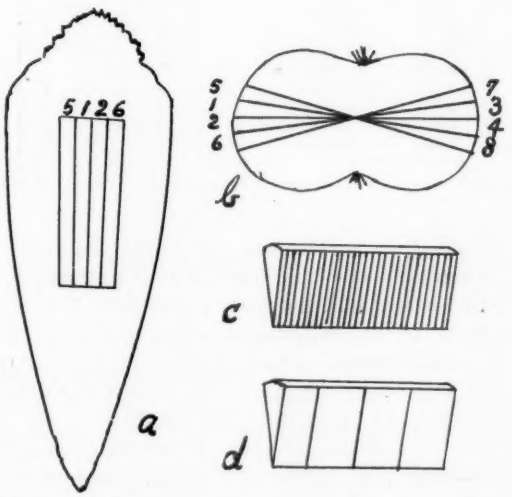

FIG. 2. Method of sampling beet and sectioning the samples.
a. Shows side view of beet indicating position of samples by number.
b. Sectional view indicating same.
c. Method of slicing wedge shaped sample into 1/16 inch slices.
d. Method of obtaining 3/8 inch slices.

The wedge-shaped pieces of beet tissue were sliced on a glass plate and weighed quickly into flasks, with precaution against evaporation. Samples 1, 3, 5, and 7 were cut into slices not over one-sixteenth of an inch thick. Samples 2, 4, 6, and 8 were cut into three-eighths inch thick slices. The

samples were treated with lead sub-acetate and then steamed under pressure. After the autoclave treatment, samples 3, 5, 4, and 6 were cooled to 20° C., made up to 100-cc. volume, let stand for four hours and then read. The flasks containing samples 1, 7, 2, and 8, which were plugged with cotton before steaming, were allowed to stand seven days and then made up to 100-cc. volume, allowed to stand four hours and then read.

The results of this experiment, as shown in table I, indicate that in the

TABLE I

THE EFFECT OF (1) THE SIZE OF BEET TISSUE PIECES IN THE SAMPLES, (2) THE PERIOD OF EXTRACTION, AND (3) THE AUTOCLAVE EXPOSURE ON THE POLARISCOPE READINGS

SAMPLE NUMBER	WEIGHT OF TISSUE	THICKNESS OF SECTIONS	EXTRACTION PERIOD	AUTOCLAVING		CORRECTED POLARISCOPE READINGS	AVERAGE	SUCROSE
				PRESSURE	TIME			
	*grams**	*inch*		*pounds*	*minutes*			*per cent.*
1	13.01	1/16	7 days	10	1	8.45		
3	12.98	1/16	4 hrs.	10	1	8.00		
5	13.12	1/16	4 hrs.	15	15	8.00	8.20	16.40
7	12.90	1/16	7 days	15	15	8.35		
2	13.09	3/8	7 days	10	1	8.25		
4	13.05	3/8	4 hrs.	10	1	7.05		
6	13.03	3/8	4 hrs.	15	15	7.25	7.65	15.30
8	13.15	3/8	7 days	15	15	8.05		

* Samples weighed to nearest centigram and readings corrected for 13 grams, weight of sample.

REED method it would be necessary to control the size of the pieces of beet tissue in the samples to obtain a semblance of uniformity of extraction. This is shown by the fact that the finely cut samples 1, 3, 5, and 7 gave an average of 16.40, while the coarser cut samples 2, 4, 6, and 8 gave an average of 15.30 per cent. sucrose. The fact that those four samples read after four hours gave an average reading of 7.58 (15.16 per cent. sucrose) and those four read after seven days an average reading of 8.28 (16.56 per cent. sucrose) indicates that the size of pieces in the tissue samples appreciably affects the time required for diffusion of the sucrose into the solution even if the pieces are autoclaved.

EXPERIMENT NO. 6.—In order to test the effect of autoclaving on ground sugar-beet pulp, the following experiment was conducted. One large sugar beet was sampled several times with the machine in order to obtain a large composite sample of uniform pulp and eliminate the errors in the percentage of sucrose due to iregular distribution of sucrose in the beet. Since some time was required to weigh out this large number of samples and there were dangers of error due to evaporation, the order of weighing out the samples was specially arranged, as shown by the sample numbers

in table II. The samples to be autoclaved were thus given the advantage and the cold water and alcohol samples were at a disadvantage in being the first and last samples to be weighed out.

These results show that steaming beet pulp in a basic lead acetate solution definitely decreases the polariscope readings. The results of a more

TABLE II

EFFECT OF AUTOCLAVING ON BEET PULP TREATED WITH BASIC LEAD ACETATE

SAMPLE NUMBER*	TREATMENT	POLARISCOPE READINGS	AVERAGE POLARISCOPE READINGS FOR EACH TREATMENT	EQUIVALENT PERCENTAGE OF SUCROSE
1	Alcohol extraction	9.17	9.16	18.32
29	"	9.16		
2	Cold water digestion process	9.21		
3	"	9.20		
4	"	9.15		
5	"	9.20		
6	"	9.20	9.19	18.38
25	"	9.22		
26	"	9.20		
27	"	9.17		
28	"	9.20		
7	Autoclaved at 15 pounds for 15 minutes with basic lead acetate	9.13		
8	"	9.11		
9	"	8.95		
10	"	9.00		
11	"	9.07	9.04	18.08
12	"	8.95		
13	"	9.00		
14	"	9.00		
15	"	9.14		
16	Autoclaved at 15 pounds for 15 minutes with distilled water	9.20		
17	"	9.17		
18	"	9.25		
19	"	9.10		
20	"	9.20	9.17	18.34
21	"	9.20		
22	"	9.20		
23	"	9.13		
24	"	9.10		

* Numerical order indicates order in which samples were weighed.

extensive experiment confirmed this point. It is worthy of note, too, that the samples steamed under pressure with basic lead acetate were shown by the MUNSON and WALKER method to have 50 per cent. more direct reducing sugars than did those given the cold water digestion process. The extraction by alcohol was proved to be complete by the alpha naphthol test.

EXPERIMENT NO. 7.—The purpose of this test was to study the effect of different exposures to steam pressure on finely ground sugar-beet pulp in distilled water. Samples were weighed out from a composite sample of machine-cut pulp, autoclaved with distilled water, cleared, made up to volume and polarized. The results are shown in table III.

These results indicate that prolonged exposures of sugar-beet pulp to steam under pressure, even when in distilled water, appreciably decreases the amount of sucrose, but that short exposures at ten pounds, or even twenty-seven pounds pressure does not cause an appreciable decrease.

EXPERIMENT NO. 8.—The following experiment was planned to determine the effect of autoclaving chemically pure sucrose with each of the following solutions: Basic lead acetate, 1.0073 sp. gr.; neutral lead acetate, 0.5 cc. in 100-cc. volume of solution; and distilled water.

TABLE III

EFFECT OF DIFFERENT LENGTHS OF EXPOSURE TO STEAM UNDER PRESSURE ON SUGAR-BEET PULP IN DISTILLED WATER

SAMPLE NUMBER	EXPOSURE		POLARISCOPE READINGS
	TIME	PRESSURE	
	minutes	*pounds*	
1	45	15	7.34
2	45	15	7.37
3	1	10	7.59
4	1	10	7.56
5	1	27	7.52
6	1	27	7.55
7 Check*	—	—	7.55
8 Check*	—	—	7.56

* Cold water digestion.

The results (table IV) indicate that pure sucrose in distilled water is slightly decomposed by steam at 15 pounds for 15 minutes and more so by prolonged exposure. They also show that in basic lead acetate solution sucrose is even more markedly decomposed by steam under pressure. Samples 16, 17, 18, and 19 were acid to litmus after autoclaving. Samples 10 and 11 reduced an alkaline copper salt solution at 70° C. in 5 minutes, while samples 16 and 17 showed marked reduction within one minute at room temperature. These results show that even pure sucrose can not be safely autoclaved with either of the lead solutions at 15 pounds for 15 minutes.

TABLE IV

THE EFFECT ON PURE SUCROSE OF AUTOCLAVING IT IN DISTILLED WATER WITH VARIED EXPOSURES AND ALSO IN BASIC AND NEUTRAL LEAD ACETATES

SAMPLE NUMBER	SOLUTION	STEAMING		POLARISCOPE READINGS
		TIME	PRESSURE	
		minutes	*pounds*	
1 Check				10.00
2 Check				9.99
3 Check				10.00
4 Check				9.99
5 Check				10.00
6	Distilled water	15	15	9.95
7	"	15	15	9.95
8	"	15	15	10.00
9	"	15	15	9.98
10	"	120	15	9.85
11	"	120	15	9.60
12	Basic lead acetate	15	15	8.90
13	"	15	15	9.28
14	"	15	15	9.15
15	"	15	15	8.85
16	"	120	15	8.10
17	"	120	15	8.65
18	Neutral lead acetate	15	15	9.67
19	"	15	15	9.73

EXPERIMENT NO. 9.—The preceding experiment was repeated using one-half normal weights (13.000 grams) of pure sucrose per 100 cc. solution, and treated, as shown in table V.

These results definitely confirm the results of experiments 6 and 8, and show that the polarization of sucrose is reduced by autoclaving at 15 pounds for 15 minutes, and strikingly so when basic lead acetate is present.

EXPERIMENT NO. 10.—The increased acidity of sugar-beet pulp due to autoclaving was demonstrated by digesting 13.000-gram samples of beet pulp in 88.5 cc. of dilute basic lead acetate, or water, and following the standard beet-sugar factory method for titration of acid solutions. In 100 cc. of filtrate from beet pulp autoclaved at 15 pounds for 15 minutes with basic lead acetate, the acidity corresponds to 0.058 gram CaO, while that for the check solution not autoclaved was only 0.044 gram CaO. In other words, 14 cc. of N/28 alkali were required to neutralize the increased accumulation of acid in the beet pulp due to autoclaving. The increased acidity of sugar-beet pulp autoclaved in distilled water at 15 pounds for 15 minutes amounted to 0.010 gram CaO, or 10 cc. of N/28 alkali. These

TABLE V

THE EFFECT OF AUTOCLAVING ON SUCROSE DISSOLVED IN DISTILLED WATER AND IN DILUTE BASIC LEAD ACETATE SOLUTION

SAMPLE NUMBER	WEIGHT SUCROSE	VOLUME OF SOLUTION	TREATMENT	POLARISCOPE READINGS AT 20° C.
	gm.	*cc.*		
1	13.000	100	Distilled water (check)	49.80
2	13.000	100	Autoclaved at 15 pounds for 15 minutes with distilled water	49.55
3	32.500	250	Distilled water (check)	49.85
4	32.500	250	Autoclaved at 15 pounds for 15 minutes with distilled water	49.55
5	32.500	250	Autoclaved at 15 pounds for 15 minutes with dilute basic lead acetate solution	47.65*

* This sample was so badly discolored that a tenfold dilution was required before reading.

results furnish an explanation of the reduced polarization of sucrose and of sugar-beet pulp after being autoclaved.

EXPERIMENT NO. 11.—This experiment was planned to determine what, if any, percentage of the sugar-beet cells in finely ground pulp retained their sucrose after the cold water extraction. A composite sample was cut by the machine from three sugar beets. Two 13-gram samples, cold water digested, read 9.45 and 9.43, or an average of 18.88 per cent. sucrose. Four other 13-gram samples were weighed out and washed repeatedly by pouring cold water over the pulp in funnels. After 24 hours the wash waters gave a negative alpha-naphthol reaction showing complete absence of sucrose. This pulp was examined under a microscope and found to be made up of aggregate of cells three or more layers thick. The washed samples were then transferred to 100-cc. sugar flasks and digested 30 minutes at 75° to 80° C. Each of these hot water extractions read zero in the polariscope and gave negative alpha-naphthol reactions. This shows that the hot water had failed to extract additional sucrose, and therefore that the cold water had extracted the sucrose completely.

As some might question the effect of the long period (24 hours) of cold water washing, the experiment was repeated. The beet pulp used tested 18.15 per cent. sucrose. Two hours were required to completely wash the three samples with cold water under ordinary pressure. Between 500 and 700 cc. of water were used for each 13-gram sample. The samples were

transferred to 100-cc. flasks and hot water digested. Each filtrate gave zero polariscope reading and all but one a negative alpha-naphthol reaction. The reaction in this one case was very faint. This confirms in general the previous test. These results show that cold water digestion is effective with finely ground pulp.

EXPERIMENT NO. 12.—In order to check the cold water digestion method with the freezing method, a composite sample of uniform pulp was cut by the machine from one beet. Three samples of this pulp after digesting with cold basic lead acetate read 8.52, 8.53, and 8.52, respectively. Five samples of this pulp were frozen with solid carbon dioxide for two hours. These samples after clearing read 8.53, 8.52, 8.54, 8.52, and 8.51, respectively. These results show that for finely ground pulp the cold water digestion method is as accurate as the freezing method.

Four comparable samples were cut with a knife from this same beet that furnished the pulp samples. The first two samples were sliced to a thickness of one-fourth inch or less, frozen as stated above, and read as 1.30 and 1.00, while the other two samples sliced to a thickness of one-sixteenth inch or less read 2.30 and 3.52, respectively. One and one-half hours were allowed for clearing and diffusion before reading. These results confirm the results of experiment 5, and show that the freezing method, as well as the autoclave method, requires a finely divided pulp. It is noteworthy that the results obtained here by the freezing method are not in harmony with the uniform results which REED obtained from 15 samples (*loc. cit.*, p. 369).

Discussion

Regarding the cold water digestion of sugar-beet pulp, the case is simple. If the pulp is ground fine enough to permit of complete extraction, no point is to be gained in autoclaving since heating under pressure is extremely likely to cause changes which materially affect the specific rotatory power. If the pulp is not fine enough for cold water extraction, the hot water or alcohol method should be used.

While some low speed rasps and food choppers cut too coarse a pulp for cold water extraction, this is not the case with the disc machine devised by the writer. The disc, which is of special design for cutting a fine pulp, runs at 1725 rpm. and cuts a pulp fine enough for cold water extraction. Extensive earlier experiments of the writer as well as those of other workers (**2, 11**), show that the cold water digestion method is, with due precautions, accurate to within one-tenth of one per cent. It is a rapid method which affords a degree of accuracy sufficient for the critical testing of beets for genetical purposes as well as for various phases of physiological research.

In general commercial practice the cold water digestion method is widely used in connection with beet-sugar factory operation and the settlement of grower contracts involving the percentage of sucrose in the beets. Here also a reasonable degree of accuracy is necessary, and, naturally, control analyses by hot water digestion are frequently run. All this procedure is clearly contradictory to REED's data and consequently to his conclusions.

The basic assumption on which REED's work was done is that the unbroken cells would not allow the sucrose in them to escape. From the experiments here reported, it appears that the plasma membranes of the unbroken cells of a fine pulp are rendered permeable to sucrose. Experiment no. 11 shows that sucrose under cold water extraction diffuses readily from finely ground pulp. Presumably this is due to certain phenomena associated with the stimulus of wounding (**5**). This suggestion regarding the probable explanation of the effectiveness of cold water extraction is worthy of special attention.

Regarding the second major point of interest—the autoclave method—it will be noted that one very important point was overlooked. Even though sucrose is relatively stable in alkaline solution (**4**), in the writer's tests pure sucrose was not stable when autoclaved at 15 pounds for 15 minutes with either distilled water, neutral lead acetate, or basic lead acetate solutions. This is in harmony with other reports, *e.g.* BROWN (**2**) quotes HERZFELD to the effect that sucrose dissolved in water is decomposed by heating. Further, the results here reported show that by exposure to high temperature and pressure, the solution containing basic lead acetate and sucrose was completely neutralized and direct reducing sugars were formed. It is a regular part of the commercial refining process to neutralize the acids in the extracted beet juice by means of lime in order to prevent losses of sucrose. The results of the writer's experiments along this line are quite in harmony with these long established facts and practices. REED says that the autoclave method yields a higher sugar reading than does the cold water digestion process and that it does not affect refined sugar when this is added to samples with lead sub-acetate before heating. The writer has shown that the autoclave method consistently and seriously decreases the sucrose reading of beet juice; that the method results in decomposition of pure sucrose, and that the polariscope reading of even pure sucrose in distilled water or lead acetate solutions is lowered by the autoclaving REED recommends for beet tissue samples.

One other point of minor interest should be mentioned. REED states that "chunk" samples are as satisfactory as pulp with the autoclave method. The writer's results show (experiments 4 and 5) that complete diffusion from such "chunks" requires days (as opposed to minutes) for

extraction if the size of the pieces in the samples are of the type REED evidently proposes for use. These statements are equally true when applied to the freezing process (experiment 12). If finely ground pulp is used, the freezing method appears to have fewer objectional features than the autoclave method, since no reduction was noted in the sucrose reading of frozen pulp. However, no good reason for the recommendation of either is evident. The practice formerly followed in the writer's work, in taking pulp samples, has been to sharpen the disc of the machine each year, but in the 1929 tests it was used the second season without sharpening. As a result, the pulp used in these experiments has not been quite so fine as the pulp used in previous investigations. The pulp cut at this time contained many shreds which have not appeared in the pulp from the newly sharpened disc. The fact that under these conditions the machine still cuts a pulp sufficiently fine for cold water digestion, very decidedly strengthens the results and arguments here presented.

An attempt to discard a long established method without an abundance of carefully confirmed supporting evidence is very regrettable. In this instance not only will it cause confusion in investigational work but it is likely also to make trouble in beet-sugar factory operations. Unless promptly corrected, the mistaken criticism of the cold water digestion method is likely to arouse questions in the settlement of grower contracts where accurate determination of the percentage of sugar in the beets is presupposed.

Acknowledgments are due to Dr. EUBANKS CARSNER for helpful suggestions and criticisms.

Summary

1. It was experimentally demonstrated that the cold water digestion of finely ground sugar-beet pulp gave higher and more consistent polariscope readings than did the comparable samples autoclaved, as recommended by REED.

2. Evidence was secured that the time required for complete extraction of sugar from autoclaved or frozen beet tissue varied directly with the size of the tissue pieces in the sample.

3. Autoclaving at 15 pounds for 15 minutes with either basic or neutral lead acetate reduced the polariscope readings of sugar-beet pulp and of pure sucrose. This polariscope reading decreased as the time of autoclaving was increased.

4. Pure sucrose was measurably decomposed by autoclaving at 15 pounds for 15 minutes with basic lead acetate.

5. The polarization of pure sucrose dissolved in distilled water was also lowered by autoclaving.

6. The results obtained show that the cold water digestion method for the determination of sucrose in sugar-beet tissue is superior to autoclave digestion, and that the cold water extraction method can be depended upon when finely ground pulp is used. It is suggested that the extraction of sucrose by cold water from unbroken living cells may be due to changes in permeability resulting from wounding of the tissue.

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ESTIMATION OF NITRATE NITROGEN IN PLANT JUICE: A STUDY OF THE EXPRESSION AND CLARIFICATION OF THE JUICE[1, 2]

DONALD E. FREAR

(WITH ONE FIGURE)

Introduction

Since the publication by GILBERT (3) of the colorimetric method for the determination of nitrate nitrogen, phosphate, and potash in plant juices, several workers have presented criticisms and modifications of this method as applied to nitrates. These include particularly HOLTZ and LARSON (7) and EMMERT (2). Considerable work has been done in this laboratory on this particular determination and the purpose of this paper is to present the modifications which have evolved here during the past four years.

The GILBERT method is essentially as follows: The fresh plant tissue is ground and the juice expressed through fine mesh cloth. This juice is decolorized with carbon black and finally clarified by the addition of solutions of $AgSO_4$, $CuSO_4$, and a mixture of solid $Ca(OH)_2$ and $MgCO_3$ as recommended by HARPER (5). It was originally the plan to determine nitrate nitrogen, phosphorus, and potash on aliquots of the original juice. As the phosphate determination does not permit the use of alkaline reagents for clarification, the treatment with carbon black was extremely important. Later it was believed that heating would coagulate much of the colloidal material, allowing its separation with the carbon black on the filter. This was adopted shortly after publication of the original method, but has never been described in the literature.

HOLTZ and LARSON (7) have published a criticism of the recovery of nitrate nitrogen by the GILBERT method, stating that, using the procedure as published, they were only able to recover 40–45 per cent. of the nitrate added to extracts of wheat plants. This extremely low recovery they attribute to the use of $MgCO_3$ in the final clarification, which prolonged the final evaporation. These workers have suggested a modified procedure

[1] Contribution no. 390 of the Rhode Island Agricultural Experiment Station.

[2] The product collected when plant tissues have been subjected to pressure has been variously termed sap, juice, plant solution, tissue fluid, expressed plant tissue fluid, etc. In order to reduce this confusion of names the author suggests that the word "juice" be used as defined in the Oxford Dictionary; "The watery or liquid part of vegetables or fruits which can be expressed or extracted." The New Webster's International Dictionary defines juice: "The extractable fluid contents of plant cells or plant structures, consisting of water holding sugars or other substances in solution."

in which the $AgSO_4$ and $CuSO_4$ solutions, the carbon black, and the $Ca(OH)_2$ are added at the same time, and filtered without heating. HOLTZ and LARSON further report that this method gives them 90–100 per cent. recovery of added nitrate nitrogen, but their data show no higher results on the original sample of extract than those obtained by the GILBERT method.

The use of carbon black has been criticized by EMMERT (**2**), who cites the theoretical possibility of reduction of nitrates in the solution in the presence of the carbon black. In addition, he mentions the difficulty in obtaining suitable carbon black, and the possibility of relatively great adsorption and occlusion with some brands.

The GILBERT method as it was originally described gave results for nitrate nitrogen, phosphorus, and potash in the juice of plants that correlated with the amounts of these elements applied to the substrate upon which the plants were growing, and with crop yields. The method has then fulfilled the requirements which were imposed upon it, and it still is to be considered sufficiently accurate to indicate any large differences which may exist in crop juices. The modified method presented in this paper includes several refinements in technic that have improved the accuracy of the method so that it more nearly measures the true quantity of nitrate nitrogen in the plant.

Modifications in the method

COMPARISON OF METHODS FOR OBTAINING THE JUICE

Considerable difficulty was experienced in obtaining sufficient juice by the grinding and straining procedure, especially from small samples. There is some question whether the juice so obtained is a representative aliquot of the juice present in the plant, and it was felt that a more suitable method for securing samples of juice was desirable. Several workers (GORTNER and HARRIS (**4**), KORSTIAN (**8**), NEWTON, BROWN and MARTIN (**12**), etc.), have used an ice-salt bath to freeze the plant tissue. This process was used in this laboratory with considerable success, but in some cases the plant tissue was extremely resistant to freezing at the temperature resulting (−10 to −15° C.). Later, following the method of HARVEY (**6**), MEYER (**11**), LEWIS and TUTTLE (**9**), and others, solid carbon dioxide was used as a freezing medium with greater success. The temperature was so low that there was no question of the thoroughness of the freezing when the tissue was placed in contact with the solid CO_2. There has recently been placed on the market a machine which manufactures small cakes of the solid CO_2 from the compressed gas in drums. This eliminates purchasing large quantities of the solid CO_2, and the cost of freezing individual samples is low.

It is unlikely that the substitution of solid CO_2 for the ice-salt bath would affect the recovery of nitrate nitrogen from the resulting juice, since MEYER (11) cites evidence that there is little difference in osmotic pressure between samples of juice expressed after freezing with liquid air, solid CO_2, and ice-salt mixture. No direct comparison has been attempted in this laboratory.

After allowing the sample of plant tissue to remain in contact with the solid CO_2 for four hours or more, it is thawed, and pressed with mechanical pressure. An especially designed press cage has been used with good results in a hydraulic press maintaining 1000 pounds per square inch pressure on the tissue.

The cage used was designed to give the greatest ease of cleaning together with the lowest cost of manufacture. Three parts make up the entire assembly: The *sleeve, A,* is a six inch length of cold drawn seamless steel tubing with an outside diameter of three inches, and one-eighth inch walls. The *base plate, B,* is machined from a three-inch cast iron disc three-fourths inch thick to a diameter one sixty-fourth inch less than the inside diameter of the sleeve, leaving a one-quarter inch flange to allow the plate to extend only one-half inch into the sleeve. The *plunger, C,* is machined from a three inch bar of cast iron, six and one-half inches long, leaving a half-inch flange at one end to facilitate removal. This plunger is machined to 0.001 inch of the inside diameter of the sleeve. (See figure 1).

The dimensions given may be varied to suit the individual requirements: A cage made from one-inch tubing two and one-half inches long is very convenient for small samples. These cages can be readily taken apart and cleaned, a convenient feature when a large number of determinations are to be made.

There is a small hydraulic press on the market which is convenient, satisfactory, and relatively inexpensive, and which is designed for the expression of juices and oils. The juice as it is expressed from the tissue flows from the lower end of the cage and into a gutter in the press plate of the press, from which it discharges into a beaker.

The physical character of the juice from the above procedure is markedly different from that obtained when the tissue is ground. The juice from frozen tissue is practically free from cell débris and contains only small quantities of protoplasm, whereas the juice from ground tissue contained much colloidal material.

CARBON BLACK AS A DECOLORIZING AGENT

The treatment with carbon black was necessary in the original preparation of the juice, since, as has been noted above, two other determina-

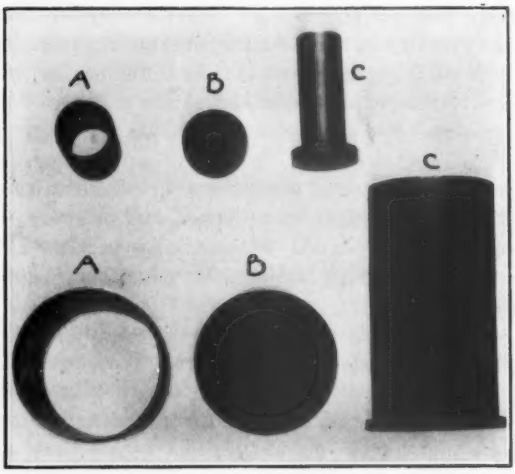

FIG. 1. Press cages used in the expression of plant juices: *A*, sleeve; *B*, base plate; *C*, plunger.

tions were to be made on aliquots of the sample. Since this work is directly concerned only with the determination of nitrate nitrogen, it was found that the reagents recommended by HARPER (5), with slight modifications, removed all of the coloring matter without the preliminary carbon black decolorization. In addition to this, there is considerable possibility

TABLE I

RECOVERY OF NITRATE NITROGEN FROM SYNTHETIC SOLUTION WHEN DIFFERENT BRANDS OF CARBON BLACK WERE USED. TWENTY-FIVE CC. OF A SOLUTION CONTAINING 100 PPM. NITRATE NITROGEN PLUS 2.5 GM. OF CARBON BLACK

BRAND	NITRATE NITROGEN RECOVERED
	ppm.
None	100
A	95
B	105
C	109
D	99
E	14

that the carbon black will adsorb or occlude some of the nitrate nitrogen present in solution. Some carbon blacks, on the other hand, contain nitrates and perhaps free nitric acid. A trial of five brands of carbon blacks is shown in table I.

The fact that the determination could be made on juices without carbon black made possible a study of the amounts actually removed from beet juices by the black itself (table II).

TABLE II

RECOVERY OF NITRATE NITROGEN FROM BEET JUICES WITH AND WITHOUT CARBON BLACK
TWENTY-FIVE CC. OF JUICE PLUS 2.5 GM. OF CARBON BLACK

SAMPLE NUMBER	PLUS CARBON BLACK	NO CARBON BLACK
1	563	667
2	526	602
3	641	735
4	379	757

These figures indicate that carbon black introduces the possibility of serious error. This agrees with the findings of EMERSON (**1**) and LIPMAN and SHARP (**10**), who worked with carbon black and animal charcoal. No attempt has been made to determine the way in which the carbon black operates to remove nitrate nitrogen.

EMMERT (**2**) states that there is the possibility of serious error through the continuation of reduction of nitrate nitrogen in the extract after the addition of carbon black. To the author the possibility of this taking place seems very slight. While there is no direct evidence to be offered to prove that no reduction takes place, table III gives several results which indicate that change in the quantity of nitrate nitrogen recovered is small

TABLE III

NITRATE NITROGEN RECOVERED BY GILBERT METHOD FROM LEAVES AFTER STANDING

CROP (LEAVES)	NITRATE NITROGEN DETERMINED ONE-HALF HOUR AFTER PICKING	TEMPERATURE OF ROOM	NITRATE NITROGEN DETERMINED IN LATER DETERMINATION	TIME ELAPSING BETWEEN DETERMINATIONS
	ppm.	*degrees*	*ppm.*	*hours*
Beet (midribs removed) ...	649	25	641	7
Beet (midribs removed) ...	58	25	70	5
Cabbage (midribs removed)	285	27	273	6.5

even though the juice is allowed to stand for a considerable time. These results were obtained by halving each leaf immediately after collection,

determining nitrate nitrogen in one-half at once and allowing the other half to remain at the temperature shown for the time indicated.

The juice is in contact with the carbon black for a very short time, probably not over five minutes in most cases. While the theoretical possibility that the reduction of nitrates may continue, due to catalytic action of the carbon black or plant reducing substances is acknowledged, it has been the experience of the author that this is so slight under normal conditions that it may be disregarded. It is to be noted that EMMERT secured considerable reduction only when he used metallic zinc with NaOH, a much more powerful reducing agent than normally occurs in plant tissues.

The use of carbon black is not advised without an extremely careful investigation of the effect of the carbon black chosen on nitrates in solution. In this procedure the use of carbon black is eliminated.

CLARIFICATION REAGENTS

The quantities of reagents used in the final clarification of the juice and removal of the chlorides have been changed considerably from the amounts recommended by HARPER in his original procedure. The quantity of saturated $AgSO_4$ solution used to precipitate chlorides has been reduced from 10 cc. to 5 cc., since it has been observed that the latter amount is sufficient to precipitate the chlorides present in normal juices. EMMERT recommends that the treatment with $AgSO_4$ be omitted when the chlorine content is below 20 ppm. It has been noted in this laboratory that most of the juices under examination contain more than the above designated quantity. Any considerable excess of silver, however, leaves a brown or black mirror when the solution is evaporated preliminary to the treatment with phenoldisulphonic acid.

GILBERT has recommended that 0.5 cc. of N $CuSO_4$ be used to precipitate the proteins present in the juice. This quantity is sufficient in most instances, but the use of 1.0 cc. of N $CuSO_4$ is more likely to satisfy all conditions. Table IV shows the results obtained when clarification reagents are omitted or substituted in various ways.

These data substantiate the findings of several workers, that some basic material is necessary to precipitate the silver and copper as the hydroxides. Undoubtedly the greater part of the clarification is brought about by these hydroxides, and it is essential to have them in the state of division which will give the maximum adsorption of coloring material. It is evident that $MgCO_3$ is not suited to be used alone in this connection, as there remained a dark residue after evaporation. Sodium hydroxide gave a finely divided precipitate of colloidal hydroxides which could not be removed by filtering. The use of $Ca(OH)_2$ proved to be as efficacious as the mixture with

TABLE IV

INFLUENCE OF AMOUNT AND KIND OF CLARIFICATION REAGENTS ON THE PHYSICAL PROPERTIES OF THE RESIDUE FROM EVAPORATION OF A FILTERED ALIQUOT AND THE FINAL COLOR DEVELOPED BY PHENOLDISULPHONIC ACID REAGENT

TREATMENT	COLOR OF RESIDUE BEFORE ADDITION OF PHENOLDISULPHONIC ACID	COLOR OF FINAL SOLUTION
2 cc. of juice made to 100 cc. final volume including:		
1. 5 cc. saturated $AgSO_4$: 0.5 cc. N $CuSO_4$ } 0.2 gm. $Ca(OH)_2$ + 0.5 gm. $MgCO_3$ }	White	Yellow
2. 5 cc. saturated $AgSO_4$	Black	Black
3. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$	Green	Black
4. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$ + 0.2 gm. $Ca(OH)_2$	White	Yellow
5. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$ + 0.4 gm. $MgCO_3$	Brown	Black
6. 5 cc. saturated $AgSO_4$ + 0.5 cc. N $CuSO_4$ + 0.2 gm. $Ca(OH)_2$	White	Yellow
7. 5 cc. saturated $AgSO_4$ + 0.5 cc. N $CuSO_4$ + 1 cc. 20 per cent. NaOH	Black	Black
8. Same as 7, but with 2 cc. 20 per cent. NaOH	Black	Black
9. Same as 7, but with 5 cc. 20 per cent. NaOH	Black	Black

$MgCO_3$. This substantiates the work of HOLTZ and LARSON, who found that the $Ca(OH)_2$ alone, was sufficient in the clarification. However, it was not found that the addition of $MgCO_3$ (although it reduced the solubility of the basic precipitant) influenced the time of evaporation, as these workers indicate.

The use of $Ca(OH)_2$ alone, to replace the mixture of $Ca(OH)_2$ and $MgCO_3$ gave slightly higher results, although the magnitude of this increase appears to be within the experimental error of the method (table V).

TABLE V

NITRATE NITROGEN RECOVERED, USING 5 CC. SATURATED $AgSO_4$ AND 1.0 CC. N $CuSO_4$ WITH 0.2 GM. $Ca(OH)_2$ ALONE AND IN COMBINATION WITH 0.5 GM. $MgCO_3$

CROP (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	REAGENTS + $Ca(OH)_2$ + $MgCO_3$	REAGENTS + $Ca(OH)_2$	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
Tomato	371	386	+15	+4.0
"	379	390	+11	+2.9
Spinach	140	147	+ 7	+5.0
"	144	148	+ 4	+2.7

From the results it is recommended that 0.2 gram $Ca(OH)_2$ be used in place of the mixture of $Ca(OH)_2$ and $MgCO_3$.

The original procedure recommended by GILBERT directs that after the addition of the clearing reagents the solution shall be heated. It seemed possible that the heating would alter the adsorption equilibrium which exists between the colloidal precipitate and the nitrates in solution. When the solution containing the clearing reagents was allowed to stand for one hour at room temperature clarification was as complete as when heated. A series of determinations was made to compare recovery of nitrate by the two treatments (table VI).

TABLE VI

NITRATE NITROGEN RECOVERED FROM JUICES. HEATING AFTER THE ADDITION OF CLEARING REAGENTS COMPARED WITH ALLOWING TO STAND AT ROOM TEMPERATURE

CROPS (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	HEAT	NO HEAT	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
	227	231	+ 4	+ 1.8
	259	333	+74	+28.6
Beet (midribs removed	323	398	+75	+23.2
	83	92	+ 9	+10.8
	270	300	+30	+11.1
	229	291	+62	+27.1
Cabbage (midribs removed)	377	321	−56	−14.8
	371	411	+40	+10.8
	286	244	−42	−14.7
Celery	360	360	0	0.0
	85	103	+18	+21.2
Mangels (midribs removed)	94	85	− 9	− 9.6
Spinach	125	140	+15	+12.0
	138	144	+ 6	+ 4.3
	296	285	−11	− 3.7
	379	393	+14	+ 3.7
	76	76	0	0.0
	92	90	− 2	− 2.2
Tomato	83	111	+28	+33.7
	138	118	−20	−14.5
	70	74	+ 4	+ 5.7
	28	36	+ 8	+28.6
	353	366	+13	+ 3.7
	185	200	+15	+ 8.2
Potassium nitrate solutions	185	199	+14	+ 7.6
	189	195	+ 6	+ 3.2
	192	183	− 9	− 4.7
Averages			+10.6	+ 6.7

The data given in this table indicate that, while the variation in the different individual determinations is great, by far the greater number of results show a higher value for nitrates when no heat is used in the clarification. Since the heating and cooling of the sample consumed considerable time, and the results indicate a less complete recovery of nitrates, it was decided to dispense with the heating.

At times a white precipitate will be formed when the solution containing the phenoldisulphonic acid is neutralized with NaOH. This may be removed, as can a brown precipitate which is rarely encountered at this point by allowing the precipitates to flocculate and filtering.

Method recommended

Place in a cheesecloth bag a sample of fresh plant tissue sufficient to yield a minimum of 10 cc. of juice, and freeze thoroughly for at least two hours. Either an ice-salt bath or solid CO_2 may be used, although the latter is preferable. Remove from refrigerating medium, allow to thaw, and press at once in any apparatus which will give sufficient mechanical pressure. It is best to have arrangements for duplication of this pressure on comparative samples. A hydraulic press with pressure gauge is the most satisfactory equipment. Collect the expressed juice, which should be free from cell residues, and pipette an aliquot (usually 2 cc.) into a volumetric flask (100-cc.).

To the juice in the flask, add about 20 cc. of nitrate-free distilled water; 5 cc. saturated $AgSO_4$ solution, 1 cc. N $CuSO_4$ solution, and 0.2 gram finely divided C. P. $Ca(OH)_2$, shaking after each addition. Shake thoroughly, make to volume with nitrate-free distilled water and filter after standing at least one hour. Discard first portion of the filtrate. Take a suitable aliquot (10 to 50-cc.) of the clear, colorless filtrate, evaporate to dryness on a water bath without overheating, and determine nitrate nitrogen by the phenoldisulphonic acid method, using NaOH to neutralize the acid according to Harper (**5**). If a precipitate forms at this point, allow to flocculate and filter.

Recovery of added nitrates by the modified method

The recovery of nitrate nitrogen by the above method was determined by adding a known amount of KNO_3 to the juices of different crops. Measured amounts of a standardized solution of KNO_3 were placed in evaporating dishes, evaporated to dryness, and the residue dissolved in a definite quantity of juice. This procedure obviated any change in the adsorption relation of the juice caused by dilution (table VII).

It is to be noted that in only three cases does the recovery exceed 100 per cent. The lowest recovery in the data is 78.1 per cent. and the average

TABLE VII

RECOVERY BY MODIFIED METHOD OF NITRATE NITROGEN ADDED TO PLANT JUICES

CROPS (LEAVES)	NITRATE NITROGEN			RECOVERY
	ORIGINALLY IN JUICE	ADDED TO JUICE	RECOVERED IN JUICE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
Beet (midribs removed)	163	400	488	86.7
	163	1400	1538	98.4
	227	50	240	86.6
	231	100	333	100.6
	231	200	398	92.3
Cabbage (midribs removed)	229	100	377	114.6
	229	200	371	86.5
	291	100	321	82.1
	291	200	411	83.7
	230	50	229	81.8
	230	100	286	86.7
	230	200	360	83.7
Mangels (midribs removed)	42	50	75	81.5
Spinach	30	400	353	82.1
	30	1000	822	79.8
	30	2000	1935	95.3
Tomatoes	76	25	90	89.1
	76	50	111	88.1
	76	75	118	78.1
	214	50	233	88.3
	214	100	296	94.3
	214	200	379	91.5
	270	100	390	105.4
	270	200	448	95.3
	270	250	513	98.7
Average				90.05

of 25 results is 90.05 per cent. This average recovery equals that of HOLTZ and LARSON (7) in one of their two trials.

Comparison of the two methods

When the GILBERT method was compared with the procedure outlined above the differences found were not consistent but were too great in some instances to be disregarded (table VIII).

The discrepancies may be attributed in part to the fact that the grinding of the sample did not accomplish a complete maceration of all the plant cells, and that probably in many cases the juice secured was not a representative aliquot of the juice of the plant as a whole. As has been mentioned previously, many workers have found that the juice obtained

TABLE VIII

NITRATE NITROGEN DETERMINED BY THE GILBERT METHOD COMPARED WITH THAT BY THE RECOMMENDED MODIFICATIONS

CROP (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	GILBERT METHOD	MODIFIED METHOD	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
	690	667	− 23	− 3.3
	641	757	+116	+18.1
Beet (midribs removed)	116	92	− 24	−20.7
	95	103	+ 8	+ 8.4
	135	95	− 40	−29.6
	148	112	− 36	−24.3
Cabbage (midribs removed)	67	105	+ 38	+57.0
Celery	183	183	0	0.0
Tomato	628	600	− 28	− 4.5
	637	607	− 30	− 4.7

by freezing and pressing the tissue has considerably different physical properties from that secured by grinding the tissue and extracting the juice under variable pressure.

In order to demonstrate that the amount of nitrate nitrogen found in the juice obtained by the grinding process was not comparable with that in the juice obtained when the tissue was frozen, samples of tomato leaves and beet leaves (without midribs) were ground as recommended by GILBERT. After extracting the juice which could be expressed by hand pressure, and centrifuging, the nitrate nitrogen was determined by the modified method. The ground residue remaining in the cloth was immediately frozen with solid CO_2, and allowed to stand overnight in the frozen state. Sixteen hours later this residue was thawed, pressed at once in the hydraulic press, centrifuged, and the nitrate nitrogen determined in the juice in the same manner. The averages of two closely agreeing determinations are given in each case:

CROP	LEAVES		DIFFERENCE
	GROUND NO_3–N	FROZEN NO_3–N	
	ppm.	*ppm.*	*per cent.*
Tomato	341	435	47.8
"	400	472	36.3
"	537	576	9.5
Beet (midribs removed)	310	411	37.7

It is evident that the discrepancies noted in table VIII may be attributed in part, at least, to the differences which may exist in the nitrate nitrogen content of the juices obtained by the two procedures. The juice obtained when the plant tissue is ground does not seem to be a true aliquot of the juice as it exists in the plant, and it is evident that the values obtained when the juice is secured by freezing will be more nearly correct. It is to be noted, however, that in the above experiments the differences between the two results are exaggerated, since the removal of a less concentrated aliquot tends to concentrate any fractions which may be removed subsequently. Hence the data shown indicate too high values for the frozen aliquot.

Summary

A considerable number of alterations have been made in the technic of estimating nitrate nitrogen in the juice of crop plants as previously recommended from this laboratory. It is recommended that the plant tissue be frozen and pressed after thawing rather than ground and squeezed through cloth by hand. The carbon black for decolorization has been omitted, and the quantities of reagents used for clearing the juice have been changed. A study of the recovery of nitrate nitrogen added to plant juices by the new method indicate rather wide variations, with the average of 25 determinations at 90.05 per cent.

The author wishes to acknowledge the extremely helpful criticisms of Mr. J. B. SMITH.

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THE EFFECT OF FERTILIZER ON THE QUALITY AND KEEPING QUALITY OF WATERMELONS[1]

W. D. KIMBROUGH

Introduction

There is a belief among many people in the South that the quality of watermelons is affected by the fertilizer treatment given the soil on which the plants are grown. Many people believe that melons from plants fertilized with nitrate of soda are not sweet. Some believe that when melons, which have been fertilized with this source of nitrogen, are eaten, the consumer is likely to be made ill. The claim is made that melons from plants fertilized with nitrate of soda can be detected by taste. Buyers often discriminate against melons which have been fertilized with nitrate of soda. The reason given is that the melons are likely to have white heart, and be of poor quality.

Manure is generally considered an excellent fertilizer for melons and was used almost exclusively while it was cheap and easily obtainable. As manure becomes scarcer and more expensive, commercial fertilizer is being used more extensively in the melon fields. This makes it important that information concerning the influence of fertilizers on watermelons be obtained. For this reason, an experiment was started at the Alabama Experiment Station, in 1926, to determine the effect of fertilizer treatment on the quality and keeping quality of watermelons.

Review of literature

BEATTIE (**1**) states that some of the melon growers' associations of Texas prohibit the use of nitrate of soda as a top dressing, as they consider that it makes the melons soft and of poor carrying quality. JONES and ROSA (**2**) state that the claim is often made that potassium, applied in manure, favorably affects the flavor, quality, or sugar content of watermelons. They further say that the literature does not afford any evidence for such belief; but that potassium is a nutrient no doubt profitably applied in some cases. STUCKEY (**3**) reports that the results obtained for one year showed that the amount or type of white heart could not be attributed to the kind of fertilizer used. Unfavorable weather conditions during the fruiting period seemed to be the chief cause of white heart. Judged by taste, melons from plots receiving different fertilizer treatments gave no indication that the quality of melons was influenced by fertilizer treatment.

[1] Work on certain phases of this problem is being continued.

Procedure and methods

Watermelons were grown on well drained Norfolk sandy loam. The location of plots was changed three out of four years to avoid serious injury from disease. The first two years the same area was used and the plots were 1/40 acre with plants 6 feet apart each way. The third year plots were 1/10 acre and the fourth year 1/20 acre with plants 8 feet apart each way. Plots were separated by 3-foot alley ways. The third year the plots were on new ground, while the other years old land was used that had not been fertilized for a number of years. The variety "Wondermelon" was grown the first year and "Tom Watson" was grown the other three years. Melons were thinned to two to a vine.

Fertilizer treatments were run in duplicate, while check plots received no fertilizer. Fertilizer treatments are indicated by N, P, and K or multiples of these. The explanation of the symbols used is: N = 200 pounds of nitrate of soda, or equivalent per acre; P = 400 pounds of superphosphate per acre; K = 50 pounds of muriate of potash or equivalent per acre. Sources of fertilizer named were used unless otherwise stated. All fertilizer, except the nitrate of soda that was applied as top dressings, was applied broadcast before planting the first three years, and the fourth year it was applied along the rows in strips about four feet wide. Nitrate of soda was applied at times varying from entire application before planting to as many as four top dressings during the season. Nitrate of soda was not applied in amounts exceeding the rate of 200 pounds per acre or less than 100 pounds per acre at one application. Top dressings of nitrate of soda were applied to some plots after many melons were approximately half grown. Fertilizer applications were so varied in rate and sources, and in the time of application of nitrate of soda, that if fertilizer treatment affects the quality or keeping quality of melons this should have been true of melons produced in this experiment.

Quality was determined by taste and by determining the sugar and moisture content of the edible portion of melons. Judged by looks, only melons that were ripe and in good condition were sampled. In taking samples for analysis a portion of the melon extending from the rind to the center of the heart was freed of seed and ground in a food chopper. Samples for sugar determinations were preserved in alcohol in the usual way. Individual melons from a plot were sampled, rather than taking a sample from a group of melons. Only melons weighing 20 pounds or more were sampled for analysis.

Moisture content was determined by drying thoroughly mixed samples of material to constant weight in a vacuum oven at 80° C. and 26 inches of mercury. Reducing sugars were determined and expressed as glu-

cose, and total sugars as invert sugar. Inversion was brought about by acid hydrolysis at room temperature. Reducing sugars were determined by the Bertrand modification of the Munson and Walker method.[2]

Keeping quality was determined by storing melons at room temperature and at 35, 40, and 50° F. The storage temperature range was in general 2° F. each way from that given.

Experimental results

Effect of fertilizer on plant growth

The plants showed a marked response in growth to the applications of fertilizer. Check plots produced plants that grew very slowly, but in some cases very good melons were eventually produced. The addition of both nitrogen and phosphorus was necessary for good growth, while the addition of potash along with these was necessary for best growth. Plants on plots receiving 2N made better growth than those on plots receiving 1N, but the addition of more than 2N made no apparent difference in plant growth. The third year of the experiment heavy rains apparently leached out nitrate of soda applied before planting to such an extent that plants got no benefit from it. Plants on manure plots made good growth but not as good as those on plots receiving 2N. Melons were a little earlier on manure and cottonseed meal plots than on plots receiving nitrate of soda as a source of nitrogen.

The response in plant growth to fertilizer treatment of the soil showed that the materials added were being utilized and indicated that if melons could be influenced by fertilizer treatment, this influence should have been obtained in melons produced on these plots.

Effect of fertilizer on quality of watermelons

No laboratory was available in 1926, so quality of melons was determined by taste only. Three watermelon cuttings were held that were participated in by many members of the station staff and a few others. Melons from plots receiving different fertilizer treatments were cut and sampled. Those tasting the melons did not know the fertilizer treatment of the plots on which the melons were grown. The results obtained showed that there was apparently as much difference in melons from the same plot as there was between melons from plots receiving different fertilizer treatments. There was considerable difference of opinion as to which melons were best

[2] Sugar content of the juice from the edible portion of several melons was determined with a Brix hydrometer and compared with the sugar content of the edible portion determined by the Bertrand modification of the Munson and Walker method. The average of determinations from nine melons showed that the Brix hydrometer method was about 1 per cent. higher than the other method.

but there was good agreement as to which melons were good, which were medium, and which were only fair in quality. Melons of good quality were produced from all treatments. The results of the watermelon cuttings indicated that the source of fertilizer did not materially influence the taste of the melons. After the tasting was over many ate large quantities of melons, including those that had been heavily fertilized with nitrate of soda, but no one became ill. During the period of four years, there was no report of melons from any plot being injurious to the consumer.

TABLE I

MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS IN 1927

FERTILIZER TREATMENT	MOISTURE		SUGAR—FRESH WEIGHT			
			REDUCING		TOTAL AS INVERT	
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Check	90.26		3.95		8.95	
	90.54		4.90		7.98	
	90.71		4.63		7.73	
		90.50		4.49		8.05
NP	89.79		4.47		8.69	
	91.43		4.40		7.30	
		90.61		4.58		8.00
NPK	90.44		4.98		8.11	
	90.84		5.00		7.89	
		90.64		4.99		8.00
NPK (N from urea)	90.44		5.10		8.00	
	—		4.59		7.90	
				4.84		7.95
NPK (K from K_2SO_4)			5.18		8.35	
	90.27		4.57		8.21	
				4.87		8.28
2N2P2K	90.33		4.53		8.18	
	90.04		3.93		8.10	
		90.18		4.23		8.14
3N2P2K	89.93		5.45		8.40	
	91.20		4.53		7.34	
		90.56		4.99		7.87
2NP2K			4.31		8.18	
	90.15		5.31		8.17	
				4.81		8.17
N2P2K	90.56		4.88		8.22	

The last three years of the work a laboratory was available and determinations were made to find the effect of fertilizer treatment on the moisture and sugar content of watermelons. At the time samples for analysis were taken the melons were tasted for quality by the writer and at least one assistant.[3] In general, the quality of melons judged by taste varied directly with the sugar content.

TABLE II

MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS IN 1928

FERTILIZER TREATMENT	MOISTURE		SUGAR—FRESH WEIGHT			
			REDUCING		TOTAL AS INVERT	
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Manure (rate of 7.5 tons per acre)	89.68		3.68		8.47	
	90.10		3.77		8.15	
	90.56		3.59		7.80	
	90.74		3.77		7.60	
	91.39		4.05		7.12	
	91.63		4.45		6.99	
	92.12		4.35		6.63	
		90.89		3.95		7.52
NPK	89.78		5.19		8.27	
	90.20		3.90		7.91	
	90.49		4.09		7.90	
	90.51		4.42		7.90	
	90.55		4.36		7.82	
	91.78		4.79		6.77	
	92.44		4.59		6.17	
		90.82		4.48		7.54
4N2P2K	89.52		4.58		8.53	
	89.52		4.26		8.37	
	89.91		3.94		8.30	
	90.38		4.31		7.78	
	90.51		3.86		7.61	
	90.80		4.45		7.48	
	91.38		4.99		7.11	
		90.37		4.33		7.88
3N2P2K	89.71		4.36		8.47	
	89.92		4.58		8.29	
	90.77		4.45		7.57	
		90.13		4.46		8.11
2N2P2K	90.28		4.36		7.89	
	90.65		4.59		7.77	
	90.41		4.45		7.47	
	91.92		4.72		6.55	
		90.81		4.70		7.42

[3] After tasting a number of melons it was difficult to make fine distinctions in quality.

Results of analyses are given on individual melons as well as averages. This is done in order that the actual variation in the melons may be seen. In this way the relation between moisture content and sugar content is

TABLE III

MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENT IN 1929

FERTILIZER TREATMENT	MOISTURE		SUGAR—FRESH WEIGHT			
			REDUCING		TOTAL AS INVERT	
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
	89.35		4.05		8.31	
	90.19		3.85		7.96	
	90.02		3.82		7.78	
Check	90.48		4.34		7.72	
	90.60		3.81		7.58	
	91.13		4.38		7.07	
		90.33		4.04		7.74
	90.03		3.53		7.91	
	89.94		3.50		7.87	
	90.44		3.86		7.53	
Manure (at rate of 6 tons per acre)	90.73		3.62		7.32	
	90.97		3.95		7.17	
	91.52		4.43		6.65	
		90.60		3.81		7.41
	89.12		3.95		8.49	
	89.69		4.00		7.87	
	89.70		3.95		7.82	
2N2P4K	90.14		4.10		7.68	
	91.68		4.17		6.56	
		90.07		4.03		7.68
	89.43		4.63		8.69	
	89.49		3.64		8.27	
2N2P4K (K from K_2SO_4)	89.85		4.69		8.19	
	89.49		3.89		8.12	
	92.05		3.69		6.26	
		90.06		4.13		7.91
	89.96		4.53		8.11	
	90.08		3.73		7.92	
2N2P	90.49		4.46		7.62	
	90.62		3.21		7.50	
	90.41		4.39		7.48	
		90.31		4.06		7.73
	89.77		4.80		8.17	
	90.30		4.11		7.92	
2N2P4K (N from C. S. M.)	91.14		4.38		7.38	
	91.02		4.00		7.35	
	90.63		4.63		7.26	
		90.57		4.18		7.62

also clearly shown. The results secured in the different years are presented separately because of seasonal differences and differences in fertilizer treatments. Results are presented only from those plots that received fertilizer treatments most likely to affect the melons. The most important comparisons are between melons from the heavily nitrated plots and those from check plots, manure plots, and plots receiving cottonseed meal as a source of nitrogen. Results are given in tables I, II, and III.

The data in tables I, II, and III show that melons having high sugar content were obtained regardless of the fertilizer treatment. Melons from plots receiving heavy application of nitrate of soda had as high sugar content as melons from check, manure, and cottonseed meal plots. The rate or time of application of nitrate had no appreciable effect on sugar content. Potash fertilizers had no apparent influence on the sweetness of melons. There was approximately as much variation in moisture and sugar content in melons receiving the same fertilizer treatment as in melons from plots receiving different fertilizer treatment.

The consistency of the inverse relationship of sugar and moisture content is interesting. The sum obtained by adding the percentage of moisture and of sugar is almost constant.

Effect of fertilizer treatment on the keeping quality of watermelons

The first two years of the experiment melons from plots receiving different fertilizer treatments were kept at room temperature. No apparent difference in keeping quality due to fertilizer treatment was found. Melons were kept as long as six weeks without rotting, but when cut the flavor was such that they were not eatable.

Sugar and moisture content of a few melons that had been stored at room temperature, were determined. The results obtained from melons that had been stored for 14 and 18 days respectively are shown in table IV.

If tables IV and I are compared it will be seen that there was a consistent decrease in sugar content and an increase in moisture content in the stored melons. The average moisture and sugar contents of similar melons sampled the days of harvest and after being held at room temperature for 14 and 18 days respectively are shown in table V.

Melons cut after being kept from one to three weeks at room temperature seemed redder in color and had thinner rinds than melons cut immediately after harvest. This might indicate that melons continue to ripen after harvest but the quality of the melons cut the day of harvest was better than that of melons kept at room temperature for several days after harvest. The sugar content has been shown to be decreased when melons are kept at

TABLE IV

MOISTURE AND SUGAR CONTENT OF MELONS KEPT AT ROOM TEMPERATURE

FERTILIZER TREATMENT	SAMPLED 14 DAYS AFTER HARVEST			SAMPLED 18 DAYS AFTER HARVEST		
	MOISTURE	SUGAR		MOISTURE	SUGAR	
		REDUCING	TOTAL AS INVERT		REDUCING	TOTAL AS INVERT
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Check	90.79	3.28	7.48	91.09	3.37	7.31
NPK	90.85	3.36	7.15	92.13	3.21	6.39
N2P2K	91.22	3.41	7.20	91.53	3.41	6.94
2N2P2K	91.69	2.84	6.65	91.93	3.36	6.65
3NPK	91.81	3.80	6.52	92.00	2.71	6.60
3N3P3K	91.68	3.80	6.82			
2NP2K	91.09	2.89	7.19			
3N2P2K	91.35	3.80	6.82			

room temperature, so it is doubtful if melons ever get sweeter after they are harvested. The increase in moisture content shown in table V is not large, but it evidently occurs. The loss of moisture due to transpiration is not enough to balance the loss in sugar content plus the moisture formed in the respiratory process.

TABLE V

COMPARISON OF AVERAGE MOISTURE AND SUGAR CONTENT IN MELONS SAMPLED THE DAY OF HARVEST AND MELONS KEPT AT ROOM TEMPERATURE

TIME OF SAMPLING	MOISTURE	SUGAR		DECREASE IN SUGAR CONTENT
		REDUCING	TOTAL AS INVERT	
	per cent.	*per cent.*	*per cent.*	*per cent.*
Day of harvest	90.47	5.09	8.03	
14 days after harvest	91.31	3.32	6.95	13.45
Day of harvest	90.69	4.47	8.20	
18 days after harvest	91.73	3.21	6.77	17.44

In 1928 and 1929 melons were stored at temperatures of 35, 40, and 50° F. There was no apparent difference in keeping quality due to fertilizer treatment.[4] The lower the temperature the better the melons kept. Melons kept for two months at 35° F., however, were in bad condition internally even though they seemed in fair condition externally. It seems that in order to store melons for long periods a temperature below 35° F. is necessary.

TABLE VI

MOISTURE AND SUGAR CONTENT OF MELONS STORED AT 40° F. FOR ONE MONTH

FERTILIZER TREATMENT	MOISTURE	SUGAR—FRESH WEIGHT	
		REDUCING	TOTAL AS INVERT
	per cent.	*per cent.*	*per cent.*
Manure	90.09	3.18	8.13
	90.60	2.59	7.69
	91.19	2.87	7.23
2N2P2K	90.22	2.80	7.89
	90.51	2.97	7.78
	90.81	3.19	7.48

TABLE VII

MOISTURE AND SUGAR CONTENT OF MELONS STORED AT 35° F. FOR ONE MONTH

FERTILIZER TREATMENT	MOISTURE	SUGAR—FRESH WEIGHT	
		REDUCING	TOTAL AS INVERT
	per cent.	*per cent.*	*per cent.*
Manure	90.61	2.42	7.58
2N2P2K	90.06	3.31	8.13
	91.39	4.37	6.44
3N2P2K	89.80	3.25	8.09
	91.41	4.25	6.95
PNK	89.93	2.92	8.08
	90.50	2.33	7.69
Check	89.27	3.33	8.62
	90.58	3.90	7.64
	90.68	3.11	7.53

[4] In 1928, symbols indicating the fertilizer treatment of the plot from which the melon came were scratched on the melons. This proved a means of entrance for decay organisms.

Moisture and sugar contents of some melons stored for a month at 35° F. and 40° F. respectively were determined. Results obtained are shown in tables VI and VII. These tables show that there is probably very little difference in the moisture and sugar content of the stored melons and freshly harvested melons, except that the reducing sugars are apparently decreased slightly. In storage, part of the reducing sugars seem to be changed to non-reducing sugars. When cut after removal from storage, the melons were lighter red than melons kept at room temperature.

Effect of fertilizer on white heart in melons

Due to the limited number of melons during the first two years that the experiment was conducted there was no special cutting of melons to examine for white heart. Most of the melons produced were cut for various reasons, however, and no white hearted melons were noted.

TABLE VIII

Melons examined for white heart and percentage found in 1928

Fertilizer treatment	Number of melons examined	Number with white heart	White hearted melons
			per cent.
Check	35	3	8.6
2P2K	10	0	0.0
N2P2K	57	3	4.9
2N2P2K	99	13	13.1
3N2P2K	83	8	9.7
4N2P2K	72	5	7.0
Manure	57	7	12.3

TABLE IX

Melons examined for white heart and percentage found in 1929

Fertilizer treatment	Number of melons examined	Number with white heart	White hearted melons
			per cent.
Check	30	6	20.0
NP2K	33	5	15.1
N2P2K	31	2	6.5
2N2P2K	25	1	4.0
2N2P4K	54	9	16.7
2N2P4K (K from K_2SO_4)	31	3	9.7
2N2P4K (N from cottonseed meal	21	2	9.5
Manure	27	5	14.8

The last two years, 1928 and 1929, a number of melons from plots receiving different fertilizer treatments was examined for white heart. Melons having a distinct white streak in the middle and otherwise ripe, were considered to have white heart. The results obtained are shown in tables VIII and IX.

There does not seem to be any correlation between fertilizer treatment and white heart in melons. There was no higher percentage of melons with white heart from plots receiving heavy applications of nitrate of soda than from plots receiving manure or from the no fertilizer plots. The fertilizer treatment seems to have little, if any, effect on the production of white heart in melons. Most of the white hearted melons were found late in the season when unfavorable climatic conditions or disease had injured the vines.

Discussion

The results of four years work on the effect of fertilizer treatment on the quality and keeping quality of watermelons have been presented. The seasons have been variable enough to cover quite a range of climatic conditions. Three different plot areas were used and while all were of a light sandy nature, and well drained, they were somewhat variable. The response in plant growth to the fertilizer applied was usually striking, and showed that the plant was making use of the fertilizer. Especial emphasis was placed on the effect of nitrate of soda on the water-melons and this was applied at such varying rates and times that the effect, if any, should have been detected. Quality of melons was determined by taste and by analysis for moisture and sugar contents of the edible portion. Melons from heavily nitrated plots were compared especially with melons from check plots, manure plots, and plots receiving cottonseed meal as a source of nitrogen. The moisture and sugar contents of 175 individual melons were determined over a period of three years. Melons were stored at room temperature and in cold storage rooms at temperatures of 35, 40, and 50° F. to determine keeping qualities.

The data presented show no apparent difference in the quality of melons due to fertilizer treatment. The sugar content of melons varied as much between melons of the same treatment as those of different treatments. Melons from plots receiving heavy applications of nitrate of soda and applications late in the season had as high sugar content as melons from manure, cottonseed meal, or check plots. Potash fertilizers did not increase the sugar content. Melons from plots receiving nitrate of soda did not cause sickness when eaten. There was no indication that white heart in watermelons was correlated with fertilizer treatment. White heart was not usually found unless dry weather or disease had injured the vines.

There was no indication that fertilizer treatment of plants affected the keeping of the melons.

In general, quality, determined by taste, agreed very well with the sugar content, the better quality melons having the higher sugar content. Sugar and moisture content varied inversely. The sugar and moisture content relationship was such, in fact, that moisture content will give a fair estimate of sugar content.

Melons kept at room temperature for periods of one to nearly three weeks showed consistently a slight increase in moisture content and a decrease in sugar content. The decrease in sugar content is largely in the reducing sugar. The rapid decrease in sugar content in melons at room temperature is a partial explanation of the difference between shipped and home grown melons which are eaten soon after harvest. The rapid decrease in sugar at room temperature is also further evidence that the supposed ripening of melons which are kept for several days in order to improve them does not occur. The Tom Watson melon is not generally praised because of its sweetness, but many melons of this variety were found to have a high sugar content.

Summary

1. On sandy soil no apparent influence of fertilizer treatment on quality of watermelons was found.

(a) Nitrate of soda did not prevent melons from having a high sugar content and being sweet.

(b) Potash did not increase the sugar content.

2. The edible portion of the watermelon is about 98 per cent. sugar and water. The moisture and sugar content vary inversely with each other.

3. After harvest the sugar content of melons kept at room temperature decreases rather rapidly.

4. Moisture content may increase slightly in melons kept at room temperature.

5. No effect of fertilizer treatment on keeping quality of watermelons was found.

6. Temperature as low as 35° F. was not effective for keeping melons in good condition for much over a month.

7. No correlation between fertilizer treatment and white heart in melons was found.

8. White heart seems to be caused by unfavorable climatic conditions or disease injury to the vines.

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THE ESSENTIAL NATURE OF BORON TO THE GROWTH AND FRUITING OF THE TOMATO

EARL S. JOHNSTON AND PAUL L. FISHER

(WITH THREE FIGURES)

A question that frequently arises in connection with elements regarded necessary for plant growth is whether or not a particular element is an essential plant food or merely a stimulating substance. Is its rôle that of a catalyzer, or is it actually incorporated into the tissues as an integral part of the plant? These questions are more applicable to such elements as manganese, copper, zinc and boron than to nitrogen, phosphorus and potassium, which have been recognized for many years as essential nutrient units. The elements of both groups have at times been considered toxic, at others as stimulating. Here again confusion exists, for the question of toxicity seems to depend upon circumstances. Thus BRENCHLEY (1) points out that "Typical nutrient salts are toxic when they are applied singly to the plant in certain concentrations, the toxic power decreasing and the nutritive function coming into play more fully on the addition of other nutrient salts." Some elements which are toxic in high concentrations contribute to increased plant growth when their solutions are properly diluted. It is apparent then that no sharp distinction can be drawn between toxic elements, and nutrient elements, or stimulating elements if such exist. The distinction is a question rather of quantity than of kind, which should be considered in connection with other factors such as the presence of other elements. BRENCHLEY (1) has divided the class of elements that are toxic in high concentrations into two groups: "(1) Those that apparently become indifferent in high dilutions and never produce any increase in plant growth; (2) Those that are either essential for growth, or at least cause increased development, when applied in sufficiently small quantities. The former group may be legitimately regarded as toxins; the latter presents more difficulty and even now their function is not settled. It is not clear whether they stimulate the protoplasm or in some way hasten the metabolic processes in the plant, whether they help roots in their absorbent work, or whether they are simple nutrients needed only in infinitesimal quantities."

Boron without a doubt belongs in the second group. Its rôle in the plant is not known although several investigators have shown that its presence is essential for the normal growth of many plants. WARINGTON (7) states, "The fact that boron can be detected in the stem, leaves, and pods of the broad bean implies that the element becomes distributed throughout

the plant after absorption; and, further, the need for the supply of boron to be maintained during the life of the plant indicates that the initial reserve of the element in the seed is insufficient for the needs of the plant, and that it is in some way fixed and not in a state of circulation." If this be true it would then appear that boron does not act in the capacity of a catalizer, but that its rôle is similar, though to a much less extent, to those of other elements classified as essential.

"The old 'nutrients'," as BRENCHLEY (1) states, "had certain definite characters in common, in that they were essential to plant growth, the growth being in a great degree proportional to the supply, a relatively large amount of the nutrients being not only tolerated but necessary." Evidence

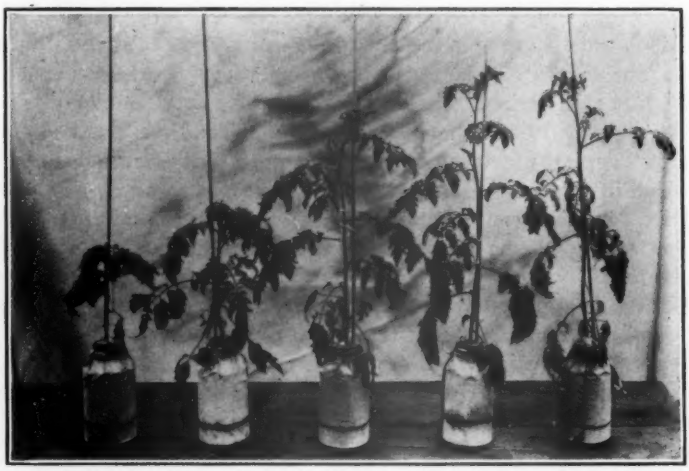

FIG. 1. Tomato plants grown in similar nutrient solutions to which different amounts of boron as boric acid had been added. (Left to right: 0.0; 0.22; 0.11; 0.55; 2.75 ppm. respectively.)

has been presented by JOHNSTON and DORE (5) which shows the possibility of a quantitative relationship existing between the amount of growth and the amount of boron present in the nutrient media. Further evidence was found in the growth of tomato plants reported by NEWELL (6) and illustrated in figure 1. An increase in concentration of boron from 0.55 ppm. to 2.75 ppm. produced a slight decrease in height in most cases. For concentrations below 0.55 ppm., with the possible exception of 0.11 ppm., a sharp decrease in height was evident. In general, these same relations also held for the green and dry weights.

Evidence is presented in the present paper which indicates the necessity for an available and constant source of boron supply in order that good

growth may be maintained throughout the life cycle of the tomato plant. Prior to these experiments it was thought that a sufficient quantity of boron, which is needed in almost infinitesimal amounts, might possibly be absorbed during the early stages of growth to amply supply the needs of the plant through the later or fruiting stage. The work of GERICKE (**3, 4**) and of BRENCHLEY (**2**) indicates that this is true for some of the recognized nutri-

FIG. 2. Tomato plants grown in similar nutrient solutions to blossoming, at which time the plant on the right was changed to a boron deficient solution.

ent elements in the case of wheat and barley. An experiment was carried out to ascertain the necessity of a continuous boron supply for the tomato plant.

Forty Marglobe tomato seedlings were set out in one-gallon culture jars, one plant per each jar, and supplied with a nutrient solution of the following volume-molecular concentration:

$Ca(NO_3)_2$	0.005
$MgSO_4$	0.002
KH_2PO_4	0.002

To this general nutrient solution manganese as $MnSO_4$ was added at the rate of 1 ppm. and boron as H_3BO_3 at the rate of 0.5 ppm. While the plants were young ferric tartrate (0.5 per cent. solution) was added daily at the

FIG. 3. Dark dead areas appear on the fruit of tomato plants grown in boron deficient solutions during the fruiting phase of growth.

rate of a cubic centimeter per liter of nutrient solution. After the roots were well developed the iron was added less frequently. The experiment was started on October 9, 1929. All the plants used were healthy and of uniform size and continued to remain so under similar treatments.

In the above solution and under similar greenhouse conditions the plants were grown until December 6. At this date three-fourths of the plants were

in blossom. These cultures were then divided into two groups and the solutions renewed. Group *A* plants were placed in a boron deficient solution while the solution of group *B* plants contained 0.5 ppm. boron the same as the original solution of all the plants. The average stem heights (usually taken at weekly intervals) for the two groups are given in table I. This

TABLE I

DATA SHOWING TOTAL STEM HEIGHT AND GREEN AND DRY WEIGHTS OF TOMATO PLANTS, EXPRESSED AS THE AVERAGE PER PLANT PER EACH GROUP

DATE OF OBSERVATION	GROUP A*	GROUP B
1929	*cm.*	*cm.*
October 17	2.5	2.4
" 24	5.0	4.6
" 31	7.4	7.3
November 7	13.7	14.0
" 14	23.5	24.3
" 22	39.5	42.3
" 29	49.7	52.9
December 7	56.2	59.1
" 16	63.5	66.5
" 22	81.1	87.1
" 29	82.2	90.8
1930		
January 3	82.7	99.8
" 17	82.2	101.3
	gm.	*gm.*
Green weight:		
Tops	143.3	146.3
Roots	52.5	59.2
Total	195.8	205.5
	gm.	*gm.*
Dry weight:		
Tops	16.8	19.5
Roots	4.3	5.4
Total	21.1	24.9

* On December 6, plants in group A were changed to a boron deficient solution while those of group B were changed to a similar solution to which boron was added (0.5 ppm.).

table also shows the average green and dry weights of the plants at the end of the experiment, January 17, 1930. It is to be noted that approximately two weeks after group *A* plants were changed to a boron deficient solution their stem-height growth began to fall off. Furthermore, the tops turned yellow and died in a manner similar to plants showing boron deficiency

symptoms at an earlier growth stage. The characteristic stem brittleness also made its appearance. The general appearance of representative plants of the two groups is shown in figure 2.

One of the interesting features of this experiment was the effect of a boron deficient solution on fruit setting and the characteristic appearance of such fruit that did develop. Approximately four times as many fruits set on the plants of group *B* as on those of group *A*. Fruits of the latter group were covered with darkened or dead areas which were apparently due to the breaking down of the cells making up this tissue. These dead areas were somewhat similar to those occurring on tomatoes injured by blossom end rot, with one exception however; the dead tissue did not necessarily begin at the blossom end of the fruit, but was scattered over the entire surface in a haphazard manner. While there was a certain similarity to blossom end rot yet the boron deficient symptom was quite different and distinct. The general appearance of representative fruits from a plant of group *A* is shown in figure 3.

It thus appears that for the tomato plant a constant source of boron is necessary for its normal growth, the setting of its fruit, and the development of its fruit. Boron is apparently fixed in plant tissues and cannot be used over and over again. It is equally important in the vegetative and the fruiting phases of growth. Boron appears to function as a simple nutrient element needed in extremely minute quantities.

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ACCUMULATED IRON IN THE NODES OF CORN PLANTS[1]

J. D. SAYRE

(WITH ONE PLATE)

HOFFER and his colleagues (**1, 2, 3**) reported on the accumulation of iron in corn plants and suggested that this had a relation to the problem of root rots. HOFFER (**4, 5**) also suggested a relation between iron accumulation and potash deficiency, and that simple colorimetric tests for accumulated iron could be used to indicate potash needs. WELTON, MORRIS and GERDEL (**8**) did not find the relation between accumulated iron and potash deficiency close enough to permit using such tests to indicate need for potash fertilizer. SALTER and AMES (**7**) found a considerable variation in the percentage of total iron in the nodal tissues among individual corn plants. They found little or no relation between such quantitative determinations of total iron and the results of the colorimetric tests used by HOFFER. HOFFER and CARR (**1**) state that the accumulated iron occurs in the phloem of the vascular bundles.

This report is an attempt to find out more fully the form or kind of iron and its place of occurrence in the corn plant. No attempt is made to correlate iron accumulation with any mineral nutrient deficiencies.

Material and methods

The corn plants used in these investigations were of such varieties as happened to be growing under conditions where iron accumulation was high. Some plants from the vicinity of Wooster were used but usually had too little iron accumulation to be satisfactory. Corn from southwestern Ohio and from the muck lands at Lodi, Ohio, constituted the best material obtainable. Plants which were "fired" usually had the largest iron accumulations, but occasional plants without this symptom would contain a considerable amount. Discoloration of the nodal tissues served as a good preliminary indicator of iron accumulation, but was not infallible, as not all such discoloration is due to iron.

In addition to gross tests on the split stalks, the iron accumulations were studied microscopically, both in cut sections of the fresh stalks and in dried, pulverized nodal tissues. The sections were cut free hand with an iron-free knife. The dried tissue examined was first pulverized and passed over a

[1] Research cooperative between the Office of Cereal Crops and Diseases of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., and the Departments of Botany and of Agronomy of the Ohio Agricultural Experiment Station, Wooster, Ohio.

300 mesh sieve. The fine material passing this sieve contained the iron accumulations freed from much of the surrounding tissues.

The potassium ferrocyanide, $K_4Fe(CN)_6$, and potassium ferricyanide, $K_3Fe(CN)_6$, tests given by KLEIN (**6**) were used. These tests are specific for iron, the one ($K_4Fe(CN)_6$) testing for ferric, and the other ($K_3Fe(CN)_6$) for ferrous iron. The blue products formed do not diffuse readily through the tissue and thus show very well the localization of the iron. The tests are more or less permanent if the mounts are properly prepared. HOFFER and CARR (**2**) used the potassium thiocyanate (KCNS) test for iron in their work. This test is very sensitive and is specific for ferric iron. The ferric thiocyanate (FeCNS) formed in the test is soluble however, and soon diffuses throughout the tissue so that localization can not be determined. Furthermore it shows the presence of ferric iron only. For these reasons it was used only occasionally to check the results of the other methods.

None of these tests show the presence of masked or organic iron, but are for the free or inorganic forms, the only kind here considered.

Observations on accumulated iron

Iron occurs in different amounts in different plants. Very few plants were found which did not show a trace of iron at some of the nodes. There was a variation in the amount of iron at the different nodes of a single plant, the amount usually being largest at the ear node. The accumulated iron was found only at the nodal plate, where the veins anastomose and extend into the leaf sheath.

Under the microscope it was found that the iron occurs in the cells of the bundle sheath and in the first layers of pith cells around the bundle. In no case was accumulated iron found in the conducting elements of the bundles. This fact is best shown by watching the section under the microscope as the test is applied. In plants which had very little accumulated iron at the nodes, microscopical examination of a thin section of the tissue before treatment with potassium ferrocyanide revealed minute granules in those cells where iron accumulates. These granules were shown to be iron or to contain iron, by their reaction as they came into contact with the test solutions. Where the iron occurs in larger amounts, the cells are found to contain either large irregular masses, which are red to reddish brown in color, or crystals which are red in color, or both. These masses and crystals gave the reaction for iron.

After standing for some time in the reagents, the masses and crystals disappear, but the blue color indicative of iron remains, diffusing throughout the containing cells. The blue substance which is formed in the reaction, however, does not diffuse through the cell wall, but eventually may be

adsorbed by the cell wall, the cell contents becoming colorless. Plate III, figures 1 and 2 show photo-micrographs of cross sections of a node of a corn plant having a large accumulation of iron. The sections are of the fresh corn stem mounted in water only, not stained for iron. The dark spots surrounding the bundles are the crystals and masses.

The crystals and masses test equally well for ferric or for ferrous iron. The masses are often as large as 30 μ and the crystals may be as large as 14–16 μ. The cells containing them are 60–80 μ in diameter.

Optical properties of the crystals and masses

The crystals and masses are isotropic; they are dark under polarized light in all positions, and remain so as the stage of the microscope is rotated. They therefore belong to the cubic crystal system. The refractive index was determined from material separated from dried, pulverized nodal tissue as already described. This material contained relatively few of the masses and crystals but enough to permit determining their refractive index by means of immersion oils. The refractive index of the crystals was 1.59 and that of the masses somewhat lower, 1.57–1.58. The crystals may be square, rectangular, or hexagonal in outline and cubic or octahedral in form (plate III, figures 3, 4, 5, 6).

Behavior of the crystals and masses with solvents

No solvent has been found which will entirely remove the iron from the tissue. The crystals disappeared in dilute acids and alkalies, in hot alcohol, in hot water, even in alcohol and glycerine in the cold after several days. The tissue which had been treated with these solvents still gave a very good test for iron, but only in those cells in which iron ordinarily occurs. Only a trace of iron could be removed by extraction with hot alcohol in a Soxhlet extractor for 20 hours. The explanation of this behavior seems to be that the substances are largely dispersed rather than dissolved. Concentrated acids and alkalies destroyed the crystals and also the whole tissue. The crystals and masses remained unaltered in tissue dried at ordinary temperatures.

Conclusion

A careful search of the literature failed to reveal a record of any compound of iron having the properties of these crystals and masses as detailed above. The problem was taken to Professor W. G. McCaughey, mineralogist of the Ohio State University, for advice. It was concluded that it is not a simple compound of iron, but probably a mixture or solid solution containing a compound of iron. The crystal itself is probably some other substance, the host substance, which contains some form of iron mixed with it in solid solution. Iron often occurs in this way, especially in organic

matter, and a trace of iron may color the host substance deeply, giving the appearance of a heavy deposit, or a pure compound of iron. Thus, no real measure of the quantity of iron present in such ways can be obtained from its color. The oxide and hydroxides of iron are the forms which usually occur in this way.

The properties of the crystals are similar to those of some of the protein crystaloids. Much more work would be needed however to permit any conclusions as to the nature of the host substance.

Summary

1. The iron studied in this work occurred only at the nodal plates of corn stems, and only in the bundle sheath and outer layers of pith cells around the bundle.

2. It accumulates as masses and crystals containing iron, which have definite optical and other properties.

3. The crystals and masses are isotropic. The crystals belong to the cubic crystal system, and have a refractive index of $n = 1.59$.

4. The iron is present probably not as a simple compound, but as an oxide or hydroxide of iron in solid solution in some host substance. The properties of the crystals suggest that the host substance may be protein in nature.

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EXPLANATION OF PLATE III

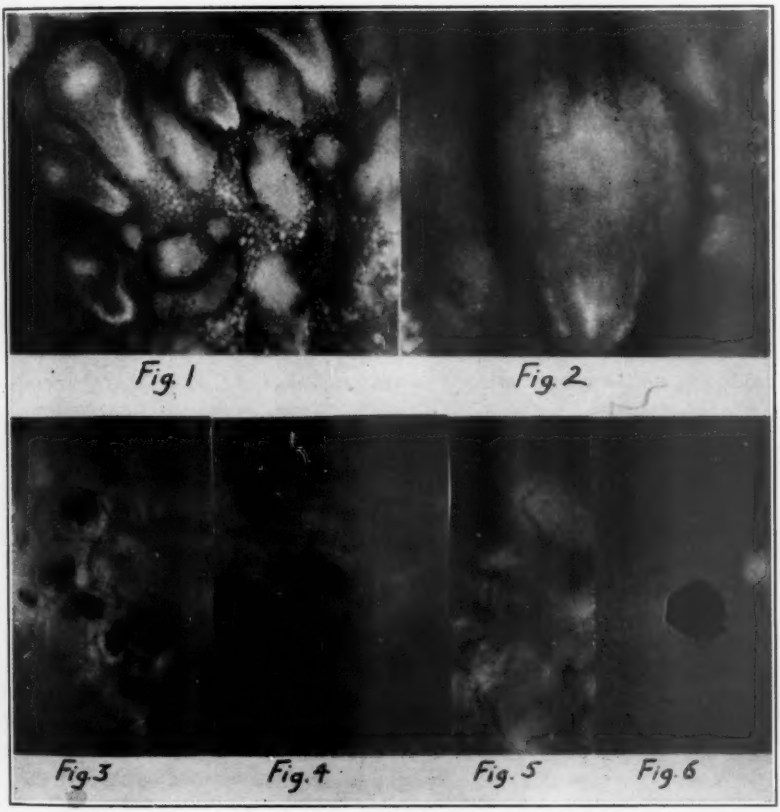
FIG. 1. Cross section of node of corn showing iron accumulated around the vascular bundles (×60).

FIG. 2. Cross section of a vascular bundle at the node of corn showing iron accumulated around the bundle (×110).

FIG. 3, 4, 5. Crystals and masses containing iron at the node of the corn stem (×260).

FIG. 6. Crystal containing iron at the node of the corn stem (×600).

All sections unstained, and mounted in water.



SAYRE—IRON ACCUMULATION

THE CHEMICAL CHANGES IN PEAS AFTER PICKING

ZOLTAN I. KERTESZ

(WITH TWO FIGURES)

It is a matter of common knowledge that unfavorable changes occur in the flavor of peas after they have been picked from the vine. "Only one hour from the field to the can" has become the watchword of the canners in their efforts to avoid these changes. It is the object of these studies to follow the changes by the determination of certain constituents of the peas.

It has been established that many parts of plants do not cease respiring upon being severed from the rest of the plant. Even though the respiration may be stopped by drying, if the moisture of the severed part is restored it will begin again. The investigations of PALLADIN showed that respiration continues even when the cells have been destroyed or killed in certain ways. This is called postmortal respiration (**8**) and an extensive literature dealing with it has already come into existence (**3**).

The chemical composition of peas at different stages of ripening was studied by BOSWELL (**4**). In his experiments it is shown that the total sugar content decreases during ripening. The starch content increases as does also the proportion of protein nitrogen. These findings are corroborated by the work of LÜNING and BEYER (**11**) who also demonstrated the increase in the proportion of starch and of protein.

There is, however, little to be found in the literature in regard to changes that may take place in peas after picking. Nevertheless, the question is of great importance, for in 1928 no less than 195,000 tons of peas were produced for canning purposes, and another considerable amount was marketed without canning. 78 per cent. of the latter were sold in New York and Philadelphia (**13**). Most of these peas must be shipped, involving a lapse of one to several days time before they can be placed in the hands of the consumer. These market peas, it should be mentioned, are left in the pods, and the changes in composition which they undergo are probably neither so rapid nor so extensive as those in shelled peas.

Work has been done on this problem with other plants, for example by ROSA (**12**) and IVANOFF and collaborators (**9**) on melons, on apples by BLACKMAN and PARIJA (**2**), etc., but no similar studies have been found for peas. They were not even included among the materials examined by BENOY (**1**). She studied the respiration of several vegetables (asparagus, lettuce, green beans, okra, green onions) harvested in edible condition, by determining the rate of carbon dioxide evolution in the first few hours after gathering. In plants where this rate is high to begin with it falls

rapidly, as in asparagus. Where the initial rate is moderate or small compared with the total observed range it falls slowly, and where the initial rate is very slight it remains almost constant for the first 25 to 30 hours. In her paper BENOY has calculated the amount of glucose corresponding to the amount of carbon dioxide produced by certain plants in the first 2 to 24 hours. This shows the amount of glucose the plant would have used up in respiration if this process had been carried on entirely at the expense of glucose present in the plant. Some of these values which will be referred to later, are as follows: asparagus, 13.68; green beans, 6.32; onions, 4.57; tomato, 2.67 gm. per 100 gm. of dry matter. LASAUSSE, GUERITHAULT and PELLERIN (**10**) determined the cellulose, total nitrogen and starch content of fresh and dried peas, but no conclusions can be drawn from this work regarding the changes in freshly picked peas.

The flavor of peas, both in pods and shelled, changed decidedly in the course of a few hours after picking. GOWEN (**6, 7**) writes that after three to four hours the flavor of peas is not as agreeable as of those freshly picked and that after eight hours they have become tougher. The observed changes in the chemical composition of peas are in all probability due to the activity of enzymes. From BENOY's experiments it may be deduced that green peas should be included among the products that show a comparatively high respiratory activity. This notion is supported by the observation of GOWEN that the temperature of peas left for 6–8 hours in a box after picking rises some 8 to 10° C.

The following experiments, which are to be regarded as preliminary, are intended to show the character of these changes. Of course, chemical analysis can not be expected to give a complete picture of the respiratory changes, but the object of these experiments was not to determine the amount of carbon dioxide produced, but to follow the changes taking place in the composition of the peas after picking. Nevertheless, the data obtained permit the drawing of certain conclusions regarding respiration.

Methods and materials

Samples of unsifted peas (variety "Rogers C") were picked at nine o'clock in the morning, taken to the laboratory, shelled by hand and weighed. The work was so arranged that none of the samples were allowed to remain in the pods more than one hour, and work was begun on them within ten to fifteen minutes after shelling. They were distributed into samples of about 30 gm. each. Two treatments were used: (1) One group of samples was placed in open dishes and left on the laboratory shelves for various periods of time. (2) Another group was macerated in a mortar, placed in an Erlenmeyer flask with 100 ml. of water containing 3 per cent. toluene, and allowed to stand for various periods of time.

After the lapse of fixed intervals of time the samples were prepared for analysis by boiling with 200 ml. of 95 per cent. alcohol for three minutes, allowed to cool, and stored in the refrigerator until the analysis was begun.

Upon removal from the ice-box the samples were ground, and the whole placed in a Soxhlet extraction apparatus and extracted with 500 ml. of 80 per cent. alcohol for several hours. The extraction was terminated as soon as a portion taken from the top of the thimble failed to give the MOLISCH (alpha-naphthol) reaction for sugar. The residue from the extraction was dried and weighed.

A 1.5 gram portion was taken for the determination of starch, and two 0.5 gram portions for the determination of protein nitrogen.[1] Reducing sugars, sucrose, and non-protein nitrogen were determined in the extract, the sugars by Bertrand's method. The procedure outlined by BOSWELL (**4**) was followed in general. His work indicated the necessity of using boiling alcohol to prevent the hydrolysis of sucrose.

Chemical changes in whole peas

The data secured on whole peas are contained in table I and figure 1. The solid lines show the changes in the peas on the vines and the dotted and dashed lines those in the peas during storage under the conditions described.

The dry matter content of the peas on the vines increases throughout the period studied.

The story of the carbohydrates as told by the chart is probably the most important. We can summarize the changes on the vine by saying that the sucrose increases rapidly for the first week after the blossoming period, remains on a level for a few days, and then decreases rather rapidly. Reducing sugars remain at a low level throughout, and starch increases gradually. The alcohol-insoluble residue which is largely carbohydrate, increases at first slowly, then very rapidly. After the peas are removed from the vines a different series of changes takes place. There is an immediate and striking reduction in sucrose in every case. On the other hand the reducing sugars and starch remain almost unchanged. The residue, however, undergoes as striking an increase as the sucrose does a decrease. The chart indicates that the two processes roughly offset each other.

In order to see to what extent this is true, the calculations in table II were made. From the per cent. of alcohol-insoluble residue was subtracted its protein (N x 6.25) and starch. The remainder is termed "polysaccharide" for convenience, although there are no doubt small amounts of fat and other substances present. It will be seen that in the first two stages

[1] The nitrogen in the 80 per cent. alcohol-insoluble residue is usually called the "protein nitrogen," although use of this term is not free from objections.

TABLE I

CHANGES IN COMPOSITION OF WHOLE PEAS KEPT IN OPEN BEAKERS AT 25°C. (ALL ANALYSES ON BASIS OF 100 GM. OF ORIGINAL FRESH MATERIAL)

No.	Age from blossom	Time after picking	Dry matter	Weight of 100 gm. original peas	Alcohol-insoluble residue	Reducing sugars	Sucrose	Total sugars	Starch	Soluble N	Insoluble N	Total N	Starch / Sugars	Insoluble N / Soluble N	Total analyzed constituents*
	days	*days*	*per cent.*	*gm.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*			*per cent.*
0	2	0	15.3	100.0	8.03	0.75	1.89	2.64	0.0	0.56	0.50	1.06	0.0	0.90	14.23
1	6	0	18.2	100.0	7.22	0.50	7.10	7.60	0.91	0.36	0.41	0.77	0.11	1.13	17.07
2		½		95.0	8.87	0.30	4.00	4.30	0.72	0.35	0.43	0.78	0.17	1.20	15.36
3		1		83.8	10.86	0.50	2.33	2.83	0.94	0.36	0.45	0.81	0.32	1.27	15.94
6		7		15.8	10.94	0.09	1.90	1.99	0.29	0.18	0.61	0.79	0.15	3.47	14.06
8		50		14.8	11.14	0.45	0.80	1.25	0.73	0.22	0.60	0.82	0.92	2.80	13.76
9	10	0	19.7	100.0	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
10 (ripe for cannery)		½		93.4	10.33	0.32	4.57	4.89	1.00	0.28	0.53	0.81	0.21	1.89	16.97
11		1		77.8	11.80	0.30	3.17	3.47	0.96	0.26	0.58	0.84	0.28	2.23	16.89
12		2		55.2	11.87	0.45	1.33	1.78	1.03	0.33	0.49	0.82	0.58	1.51	15.71
13		4		34.1	11.65	0.87	1.17	2.04	0.65	0.44	0.45	0.89	0.32	1.03	16.44
23	17	0	33.1	100.0	23.25	0.17	2.93	3.10	3.37	0.11	1.30	1.41	1.09	11.66	27.04
24		1		72.5	27.58	0.11	1.47	1.58	3.22	0.11	1.29	1.40	2.03	11.47	29.84
26		4		43.7	28.12	0.07	1.13	1.20	3.58	0.09	1.36	1.45	0.29	15.88	29.88
27		46		36.3	26.80	0.31	1.03	1.34	3.35	0.11	1.30	1.41	0.25	12.07	28.83

* = alcohol-insoluble residue + total sugars + (sol. N x 6.25).

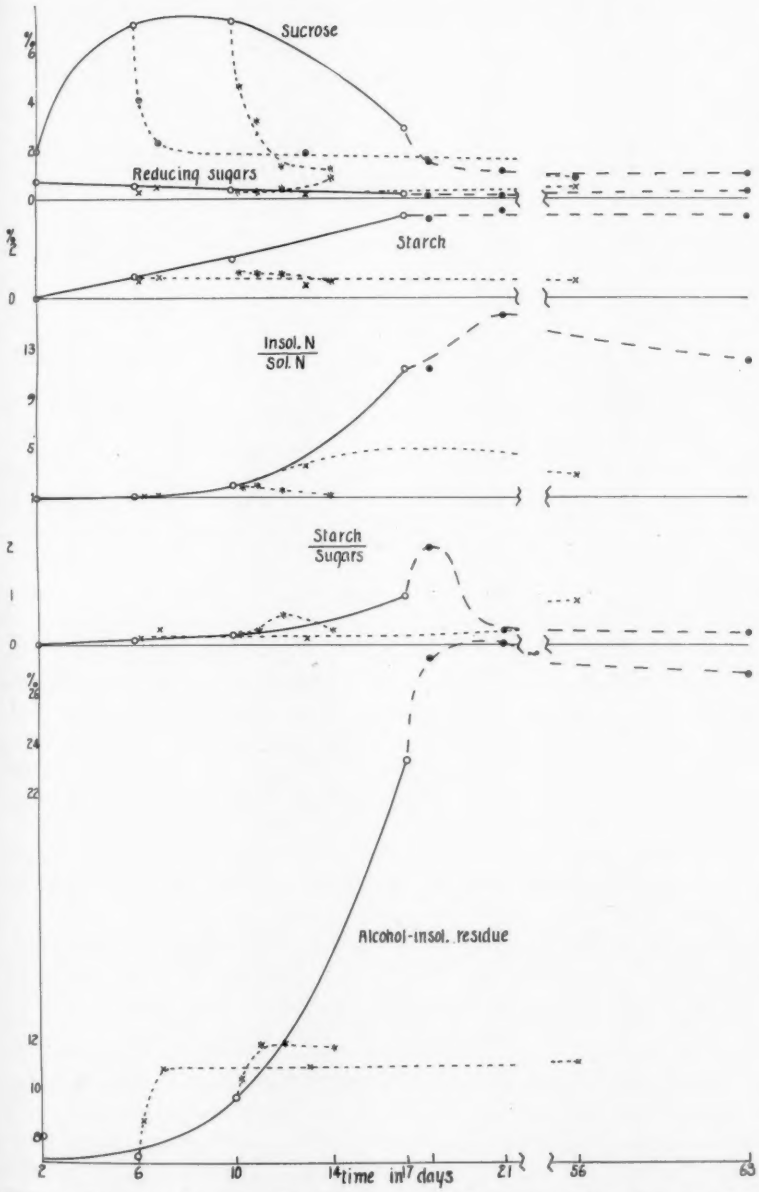


FIG. 1. Changes in the composition of whole peas after removal from the vines. Calculated on basis of 100 gm. of fresh material.

TABLE II

RELATION BETWEEN SUCROSE AND "POLYSACCHARIDE" IN PEAS AFTER REMOVAL FROM THE VINES

No.	Age from blossoms	Time after picking	A Alcohol-insoluble residue	B Polysaccharide*	C Increase in polysaccharide	D Decrease in sucrose	E $100.\frac{C}{D}$
	days	*days*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
1	6	0	7.22	3.75	.....	.....	.....
2		¼	8.87	5.46	1.71	3.10	55
3		1	10.86	7.11	3.36	4.77	70
6		7	10.94	6.84	3.09	5.20	60
9	10	0	9.66	4.74	.....	.....	.....
10		¼	10.33	6.02	1.28	2.66	46
11		1	11.80	7.22	2.48	4.06	59
12		2	11.87	7.78	3.04	5.90	51
13		4	11.65	8.18	3.44	6.06	61
23	17	0	23.25	11.76	.....	.....	.....
24		1	27.58	16.30	4.54	1.46	310
26		4	28.12	16.04	4.28	1.80	240

* "Polysaccharide" = Alcohol-insoluble residue − (protein + starch).

the decrease in sucrose can account for a large part of the increase in the "polysaccharide" phase of the residue. In the last stage it cannot begin to account for it since the increase in "polysaccharide" is three or four times as great as the decrease in sucrose. We have no explanation to offer for this. Of course, we are merely surmising that the sucrose is converted into the "polysaccharide," since there is no direct proof for it.

The chief significance in these data is the fact that sucrose is lost so quickly after the peas are picked. This partly explains rapid deterioration of quality in peas.

In order to show the changes in composition of peas during growth, part of the above data were calculated on a dry matter basis and assembled in table III. These are now directly comparable with BOSWELL's tables (**4**).

In the column headed "weight of 100 gm. of original peas" it will be seen that by the seventh day after picking the weight is less than that of the dry matter present originally. The moisture content of the stored samples could not be determined because of lack of material. This loss in material is no doubt due to respiration, and probably can account for that portion of the sucrose which was not converted into "polysaccharide."

TABLE III

CHANGES IN CHEMICAL COMPOSITION OF PEAS ON THE VINES. VARIETY: "ROGERS C." DRY MATTER BASIS. SAMPLES TAKEN IMMEDIATELY AFTER PICKING

No.	AGE FROM BLOSSOM	DRY MATTER	ALCOHOL-INSOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH / SUGARS	INSOLUBLE N / SOLUBLE N
	days	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*		
0	2	15.3	52.60	4.90	12.36	17.26	0.00	3.66	3.27	6.93	0.00	0.90
1	6	18.2	39.68	2.75	39.00	41.75	5.00	1.98	2.25	4.23	0.11	1.13
9	10	19.7	49.05	2.23	36.85	39.08	8.17	1.32	2.69	4.01	0.21	2.04
23	17	33.1	70.21	0.51	8.85	9.36	10.18	0.33	3.93	4.26	1.09	11.86

Chemical changes in macerated peas

In order to obtain some idea of the enzymes which are active in peas, the composition was determined of some samples that had been ground and had been standing under water for various lengths of time in the presence of toluene. These samples had been taken in the manner described above, and were of the same size, 30 gm. They were ground at once and covered with 100 ml. of water and 3 per cent. of toluene. After the samples had stood for stated intervals they were made up with 95 per cent. alcohol to 80 per cent. of alcohol, placed in the extractors and extracted as has been described for the other samples. The data are presented in table IV and in figure 2.

From the chart we see that the sucrose and reducing sugars are in striking contrast to their behavior in the whole peas. In the macerated peas the decrease in sucrose is accompanied by an increase in reducing sugars. This means, no doubt, that sucrose is very active. In the same way, the starch decreases, indicating the action of diastase. The protein-non-protein ratio also decreases, which must mean that proteases are functioning. The alcohol-insoluble residue shows no appreciable change in the first set of samples, and only a slight increase in the second.

In table V are presented calculations on the relation between the changes in sugars and in "polysaccharide" in these samples. The calculations are similar to those in table II for whole peas, except that in the present case the content of total sugars was considered instead of that of sucrose. The results are irregular in trying to account for the decrease in sugars by a concomitant increase in "polysaccharide," but they indicate in the first set of samples that the lost sugars can roughly account for the "polysaccharide." In the second set, the lost sugars can account for only from one-fourth to two-thirds of it. Again it is not apparent what materials do go into the formation of the "polysaccharide."

The last column in tables I and III contains the sum of all analyzed constituents. In the 6 and 10 day samples in both cases there is a marked drop in these constituents during the first few hours. This must represent loss of material by respiration, even in the macerated material.

In the 17-day samples, however, there is an increase in total analyzed material during the first few hours. This can only mean that in the fresh material there is some constituent soluble in 80 per cent. alcohol which is not accounted for in the analysis, but which later is converted into some material which is accounted for. No suggestion is offered as to what this material may be.

BROWN (5) made similar analyses of peas which had been stored in various kinds of paper wrappers. He states his results as follows: "Peas

TABLE IV

CHANGES IN COMPOSITION OF MACERATED PEAS KEPT AT 25°C. FRESH WEIGHT BASIS

No.	AGE FROM BLOSSOM	TIME AFTER MACERATION	DRY MATTER	ALCOHOL-INSOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH / SUGARS	INSOLUBLE N / SOLUBLE N	TOTAL ANALYZED CONSTITUENTS*
	days	*hours*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*			
9 (ripe for canning)	10	0	19.7	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
14		3	.....	9.96	0.27	5.00	5.27	0.78	0.34	.....	.....	0.15	.....	17.35
16		24	.....	10.53	1.03	4.27	5.30	0.63	0.38	0.42	0.80	0.12	1.10	18.20
17		48	.....	8.76	2.42	2.17	4.59	0.76	0.48	0.28	0.76	0.16	0.58	16.35
18		96	.....	8.73	5.49	1.10	6.59	0.62	0.51	0.27	0.78	0.09	0.53	18.51
23	17	0	33.1	23.25	0.17	2.93	3.10	3.37	0.11	1.30	1.41	1.09	11.66	27.04
28		24	.....	24.60	0.56	1.43	1.99	2.74	0.34	1.02	1.36	1.37	3.01	28.72
29		48	.....	25.60	0.75	0.83	1.38	2.92	0.38	1.03	1.41	2.11	2.70	29.36
30		96	.....	25.40	1.20	0.60	1.80	1.21	0.45	0.97	1.64	0.67	2.16	30.01

* = Alcohol-insoluble residue + total sugars + (sol. N x 6.25).

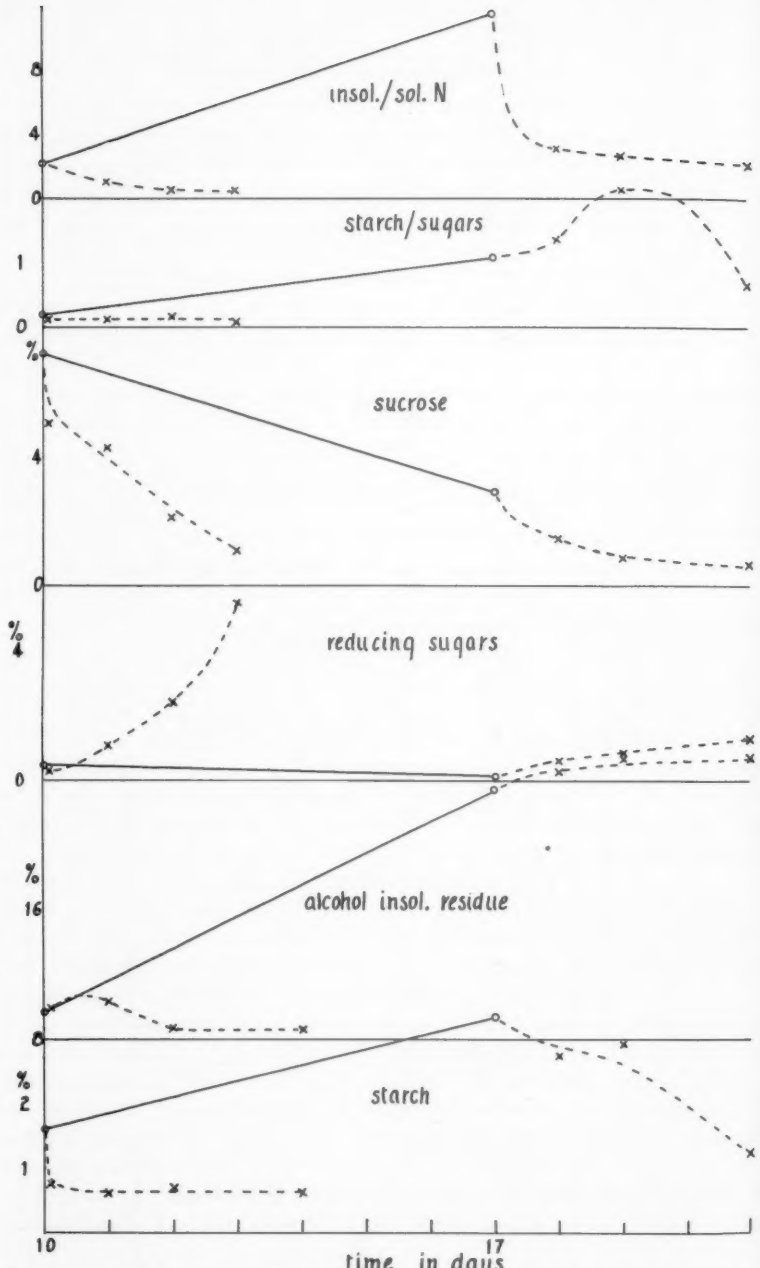


FIG. 2. Changes in the composition of macerated peas. Calculated on basis of 100 gm. of fresh material.

TABLE V

RELATION BETWEEN TOTAL SUGARS AND "POLYSACCHARIDE" IN PEAS AFTER MACERATION

No.	AGE FROM BLOSSOMS	TIME AFTER PICKING	A ALCOHOL-INSOLUBLE RESIDUE	B POLYSACCHARIDE*	C INCREASE IN POLYSACCHARIDE	D DECREASE IN TOTAL SUGARS	E $100.\frac{C}{D}$
	days	*days*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
9	10	0	9.66	4.74	.....	.....	.....
14		$\frac{1}{8}$	9.96	.....	.....	.....	.....
16		1	10.53	7.27	2.53	2.37	107
17		2	8.76	6.25	1.51	3.08	49
18		4	8.73	6.42	1.68	1.08	155
23	17	0	23.25	11.75	.....	.....	.....
28		1	24.60	15.48	3.73	1.11	336
29		2	25.60	14.24	2.49	1.72	145
30		4	25.40	18.11	6.36	1.30	490

* "Polysaccharide" = Alcohol-insoluble residue – (protein + starch).

which contained 18.6 per cent. sugar (as invert), at the start contained only 2.1 to 2.8 per cent. at the end of seven days storage at high temperatures, while those held at 32° F. still possessed most of their original sugar (60–80 per cent.). Apparently the sugar was first converted into acid hydrolyzable material, as is shown by the cold storage lots where only a partial sugar loss is shown, and later to starch, as is shown by the large amount in the high temperature lots, where the starch content has increased 400 per cent., though the acid-hydrolyzable material is but little greater than in the cold storage lots. The dry matter content, of course, varies with the storage conditions."

Thus his results and conclusions coincide very well with the present work, although here the "polysaccharide" was assumed to exist, and was not determined by acid hydrolysis.

It is obvious that both in whole and in macerated peas hydrolysis of carbohydrates and respiration are going on simultaneously. In the whole peas respiration predominates, and reducing sugars do not accumulate. In the macerated, hydrolysis predominates, and reducing sugars do accumulate. These data do not answer the question whether sucrose can be respired directly, or whether it must first be hydrolyzed.

The amount of 80 per cent. alcohol-insoluble residue increases as the peas are kept. In the immature peas (picked six days after blossoming) this increase during the first twenty-four hours is 50 per cent., in the ripe

TABLE VI

EFFECT OF FREEZING ON CHEMICAL COMPOSITION OF PEAS AT CANNING STAGE
FRESH WEIGHT BASIS

TIME AFTER PICKING	TEMPERATURE*	ALCOHOL-INSOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH / SUGARS	INSOLUBLE N / SOLUBLE N	TOTAL ANALYZED MATERIAL
hours	*deg. C.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*			*per cent.*
0	– –	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
24	– 20	11.20	0.44	4.57	5.01	1.46	0.31	0.55	0.86	0.29	1.80	18.15
24	+ 25	11.80	0.30	3.17	3.47	0.96	0.26	0.58	0.84	0.28	2.23	16.89

* Treatment.
– – = Analyzed immediately.
– 20 = 24 hrs. at – 20° C.
+ 25 = 24 hrs. at + 25° C.

peas (picked ten days after blossoming) it is 22, and in the overripe peas (picked seventeen days after blossoming) it is 18.5 per cent. In the macerated samples this residue increases at first and decreases later. The present experiments do not offer any explanation for this phenomenon.

Effect of freezing on composition of peas

It is reasonable to suppose that the rate of respiration will be lower at lower temperatures. To test this a sample of the peas picked ten days after blossoming was kept in a freezing room at a temperature of –20° C. for twenty-four hours. The results of the determinations made upon these peas are presented in table VI. For the purposes of comparison the results of the determinations upon these and upon a similar lot freshly picked are set side by side.

The low temperature has not checked the respiration as much as might have been expected. This is probably explainable by the slow cooling of the samples in the freezing room or to the very intensive respiration during the time required for the operations of sampling. The effect of low temperatures on the chemical changes in peas requires further study.

Conclusions

Definite changes in the chemical composition of shelled peas begin to take place immediately. The most important and striking of these is the decrease in the sucrose content. This partly explains the change in the flavor. The percentage of alcohol-insoluble residue increases, possibly at the expense of sucrose.

The changes in the composition of the peas which are presumably related to the respiration are interrupted when the peas become dry.

When freshly picked peas are macerated and kept under toluene-water, sucrose and starch decrease, reducing sugars increase, and non-protein N increases. This indicates that sucrase, diatase, and protease are present and active.

Even when freshly picked peas are placed at – 20° C. for 24 hours there is marked evidence of change in composition, probably because of rapid changes during the brief period before freezing actually takes place.

These experiments show in definite terms the necessity of cooking or canning peas immediately after shelling.

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A METHOD FOR THE RAPID DETERMINATION OF PHOSPHATE IN FRESH PLANT TISSUES[1]

E. M. EMMERT

Little work has been done on the distribution of phosphate phosphorus in living plants. The colorimetric method worked out by FISKE and SUBBAROW (**2**) for phosphate in animal substances should be equally good when applied to an appropriate solution from plant tissues. Preliminary trials showed that the solution of plant tissue must not be made alkaline, since phosphate was precipitated in alkaline solution. However, satisfactory solutions were obtained by using a weak acid solution and a suitable quantity of powdered charcoal or carbon black. The procedure and results follow.

Preparation of standard solution and checking of reagents

Make up a standard solution by dissolving 0.1755 gm. potassium dihydrogen phosphate in a liter of water. Five cc. of this solution contains 0.2 mg. of phosphate phosphorus. Add the same amount of acid to it as in the unknown, and neutralize just as is done in the procedure. A blank should also be prepared to check the reagents, and one standard should be treated with 1 per cent. acid and charcoal just as the unknown to test for absorption of phosphate by the charcoal, since different grades vary in their absorptive power (**2**). If the charcoal being used causes absorption, a grade should be obtained which does not. If the charcoal contains phosphate phosphorus it should be digested with 5 per cent. by volume sulphuric acid for one-half hour and leached with 1 per cent. by volume sulphuric acid until the leachings give no test for phosphates by the FISKE and SUBBAROW method, as directed below.

Procedure

Triturate thoroughly enough green plant tissue to give 0.1 to 0.3 mg. phosphate phosphorus (usually 1 gram of tissue), in a mortar, with 5 gm. of fine "decolorizing charcoal."[2] Now add exactly 50 cc. of 1 per cent. by volume sulphuric acid and mix well with the black paste. Allow to stand 5 minutes and filter. If not clear and colorless, treatment with more charcoal will be needed. The amount of charcoal used in trituration should be regulated according to the amount required to give a clear solution. Three to five grams was found to be sufficient for a one-gram sample. When the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Decolorizing charcoal from J. T. Baker Chemical Co. was used.

solution is cleared, add a few drops of phenolphthalein to an aliquot (usually 25 cc.) and bring just to neutrality with dilute sodium hydroxide.

Make the neutral solutions of the unknown, blank, and standard up to about 70 cc. and add 10 cc. of 2.5 per cent. ammonium molybdate made up in 5 N sulphuric acid. Mix and add 4 cc. of 1, 2, 4-amino-naphtholsulphonic acid, prepared as directed by FISKE and SUBBAROW (1). Mix and make up to 100 cc. After ten minutes read in a colorimeter and calculate the phosphate phosphorus in the original plant tissues.

Results

Table I shows excellent duplication. The duplicates were taken from

TABLE I

PHOSPHATE PHOSPHORUS IN GREEN PLANT TISSUES
(1-GRAM SAMPLES WERE TREATED WITH 5 GRAMS OF PHOSPHATE-FREE CHARCOAL AND 1 PER CENT. SULPHURIC ACID)

TISSUE	SAMPLE NO.	DET. 1	DET. 2	AVERAGE	ERROR FROM AVERAGE
		ppm.	*ppm.*	*ppm.*	*per cent.*
Tomato	1	480	488	484	0.8
Tobacco	1	109	109	109	0
Lettuce	1	131	131	131	0
"	2	344	344	344	0
"	3	296	280	288	2.8
"	4	342	342	342	0
"	8	448	448	448	0
"	11	416	400	408	2.0
"	17	704	672	688	2.3
"	18	448	448	448	0

the same leaf by selecting a large leaf and using tissue between the large veins. In this way uniform samples were obtained. The different lettuce samples were from plots which varied greatly in treatment. No. 17 had large applications of 85 per cent. syrupy phosphoric acid which explains its high phosphate phosphorus content. No. 3 was from a plot high in lime which probably explains the smaller amount of phosphate phosphorus obtained. No. 1 was a fresh compost not comparable with the soils in the other plots.

Table II shows conclusively that the charcoal used did not absorb phosphate from the acid solutions used.

Choice of reducing agent

TRUOG and MEYER (3) used the method of DENIGÈS in which stannous chloride is employed as a reducing agent. Their tests, however, show that

TABLE II

RECOVERY OF PHOSPHATE IN ACID SOLUTIONS FILTERED FROM PHOSPHATE-FREE CHARCOAL (TREATED WITH THE SAME REAGENTS AS WERE USED ON THE PLANT TISSUE)

TISSUE	P ADDED	P RECOVERED	ERROR FROM AVERAGE
	mg.	*mg.*	*per cent.*
1	0.185	0.185	0
2	0.185	0.185	0
3	0.185	0.185	0
4	0.037	0.037	0
5	0.370	0.370	0

the range of acidity and concentration of molybdate in which accurate results are obtained, is very narrow. If the acidity is weak the molybdate gives a color in the absence of phosphate; and if it is too strong, the blue color fails to form even if phosphate is present. It is also difficult to maintain a uniform solution of stannous chloride, a layer of mineral oil being recommended to preserve it. The solution of 1, 2, 4-amino-naphtholsulphonic acid will keep at least a month without any special treatment. It does not have such a narrow range of acidity in which it is accurate and never causes a color to form when phosphorus is not present, as long as the solution is acid and cold. On boiling, however, a blue color is produced without phosphorus. Trials with different degrees of acidity, from neutral to very acid, in the cold, produced no color from molybdate alone. Stannous chloride acts on molybdate in the cold even if phosphate is not present, unless a certain acidity is maintained, and small increases in temperature may cause a color even if the acidity is right.

Another advantage of the amino-naphtholsulphonic acid reagent is that it is not so sensitive to interference by the presence of ferric iron as stannous chloride. TROUG and MEYER (3) found that the limit with stannous chloride was 2 ppm. of ferric iron. Table III shows that as much as 30 ppm. of ferric iron may be present in the solution with 1, 2, 4-amino-naphtholsulphonic acid without interfering.

It is true that stannous chloride brings the color faster than the amino-naphtholsulphonic acid reagent, but as long as the standard and unknowns are treated alike as to time and reagents accurate results are obtained as shown by tables I and II. Interference by silica was not a factor in the plant solutions, since silica would not be put into solution by the weak acid used, even if the plant contained a significant amount.

TABLE III

EFFECT OF FERRIC IRON ON THE BLUE PHOSPHATE COLOR
(FERRIC SULPHATE WAS USED)

Fe	P added	P recovered	Notes on color
ppm.	*mg.*	*mg.*	
5	0.20	0.225	Blue matched well
5	0.20	0.20	" " "
10	0.20	0.20	" " "
10	0.20	0.20	" " "
20	0.20	0.20	" " "
30	0.20	0.20	Blue, slight cloudiness
40	0.20	0.20	Blue, cloudy
50	0.20	0.12	Slight green, quite cloudy
60	0.20		Greenish
70	0.20		Very green

Titanium and arsenates cause errors if present (3), but would not likely be present in significant amounts in plant extracts. TRUOG and MEYER (3) also found that nitrate, if present at the rate of 200 ppm. of nitrogen, caused a fading of the color. However, it would be highly improbable that a one-gram sample of any plant in 100 cc. would cause a concentration of 200 ppm. of nitrate.

Summary

1. A method of determining phosphate phosphorus in fresh plant tissue is outlined. The tissue is extracted with dilute sulphuric acid and cleared with phosphate-free powdered charcoal or carbon black. The method of FISKE and SUBBAROW is used on the cleared extract.

2. Data are presented which show good duplicates on tomato, tobacco and lettuce leaves.

3. No adsorption of phosphate by the charcoal used could be detected.

4. Preference is given to 1, 2, 4-amino-naphtholsulphonic acid over stannous chloride as a reducing agent, because:

(1) The reagent keeps better.

(2) It does not require as narrow a range of acidity for accuracy.

(3) A blue color does not develop in the absence of phosphate at any degree of acidity used in these experiments.

(4) Larger quantities of iron may be present in the tissue extract without causing interference.

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THE ASKENASY DEMONSTRATION

CHARLES H. OTIS

In the third edition of Palladin's Plant Physiology (pp. 147–150, 1926), LIVINGSTON calls attention to the fundamental and far-reaching importance of liquid tension in plants, and laments the fact that it is rarely demonstrated in university or college courses in either physics or plant physiology. There is contained in this textbook a brief account of the precautions that seem to be necessary in order to assure the success of the ASKENASY demonstration. This was followed by a paper[1] giving additional information on a simplified technique, together with a request that other experimenters record their successes or failures with the demonstration.

The writer has experimented off and on with the ASKENASY demonstration during several years. It is not only fascinating to try to better former traction records, but trial after trial leads one to better comprehension of the various forces operating and to appreciate more fully the phenomena existing in the living plant. Various procedures have been tried out and their effects have been carefully noted. Different membranes have been used, in the attempt to find one more nearly comparable with the natural plant membrane. While the experiments leave much to be desired, they have been attended with sufficient success and with such regularity that a traction experiment has been made a regular demonstration in the course in plant physiology at this university, and the apparatus is kept set up so that it may be used from year to year.

The first apparatus was set up in the manner illustrated in PALLADIN, using a single five-foot length of glass tubing with a one-millimeter capillary, a standard thick-walled atmometer cup, and a filter pump of twenty-one pounds capacity for suction. As typical of results one is likely to obtain at first, eleven trials gave the following in centimeters of rise of mercury column above mercury level in lower reservoir: 30.5, 81.3, 80.0, 63.8, failure, 100.3, 100.0, 107.5, 99.7, 103.6, failure. Uncorrected barometric pressure for this series varied between 72.9 cm. and 74.1 cm. It should be further noted that in the sixth trial the meniscus in the cup apparently broke at 95.2 cm., the level fell about 2 mm., and then started back, giving a total rise of 100.3 cm.; and again, in the eighth trial the column fell over 1 cm. at 103.5 cm., followed by recovery, and making a final height of 107.5 cm. In the many experiments subsequently performed, this phenomenon was observed on only one other occasion. The cause has been referred to as a breaking of one or more menisci, but since the cup is opaque and anything

[1] LIVINGSTON, B. E. and LUBIN, GRACE. The ASKENASY demonstration of traction transmitted through liquid water. Science n.s. 65: 376–379. 1927.

taking place in it is invisible, the phenomenon is a matter for conjecture; and some slight readjustment of parts, magnified by the small bore of the capillary, may be the explanation. The dropping of the mercury column was sudden, and if it was due to breaking of the meniscus, the method of recovery is a matter of considerable importance; for, as pointed out by LIVINGSTON, the water system usually breaks, and the mercury column then falls quickly to a height corresponding with the current reading of the barometer. LIVINGSTON further states that the system breaks, sometimes in the water in capillary or cup, and sometimes in the mercury. In our experiments, always using distilled water, but often exposing it in the boiling cup to the atmosphere for days before a trial was made, breaking of the system was never observed to take place (in the trials which were at all successful) save in the porous cup.

As the desirability of a longer capillary tube became apparent, the idea of joining two lengths of tubing together was suggested. Various kinds of bonds were tried, all without success. Finally, a method was perfected for fusing two glass tubes together, using a blow pipe and infinite patience, since it is easy to draw the capillary. The operation has been successfully applied on several subsequent occasions, but it is not recommended to the average glass blower. Later, it has been learned that glass tubing may be obtained in longer lengths on special order from the manufacturer. This would be more satisfactory, but might entail some months of waiting. Hard glass would probably be better than soda-lime glass. One set-up, made from soft glass, gave a great deal of trouble due to numerous minute holes developing successively in the upper bend of the tube after a short period of use.

The construction of a suitable evaporation membrane has been the subject of much thought and experimentation. The porous cup constitutes an artificial leaf, just as the capillary tube takes the place of a single vessel. In a living leaf the place where water loss takes place is not essentially through the stomata, but internally from the moist surfaces of the cells which abut upon the intercellular spaces back of the stomata. The walls of these cells and the plasma membranes within are not the porous structures that clay cups are. Ideally, the nearer the porous cup approximates the physical conditions of the evaporating mechanism of the living leaf, the more successful should be the demonstration as measured in lifting-power. The difference in behavior of a larger-pored cup as contrasted with a finer-pored condition (meaning large menisci as against small menisci) was demonstrated, as follows. A thin-walled unglazed porcelain cup was selected, which was boiled in sodium hydroxide and carefully washed; the rubber stopper was new and also properly treated to remove air and sulphur. Five separate trials with this cup gave three failures and the maximum rise ob-

tained was 29.2 cm. of mercury. The cup was then removed and a copper-ferrocyanide membrane was deposited in the walls by the ordinary procedure. Seven trials with the treated cup gave uncorrected results in centimeters as follows: 91.7, 96.0, 91.7, 91.0, 80.0, 88.2, and 58.0, the barometric pressure varying from 72.5 cm. to 75.0 cm. Similar thin-walled cups were used, but impregnated with a very dilute solution of cellulose acetate (Cellon, Paris) in acetone. Results were as follows, in centimeters of mercury column: 57.0, 62.0, 79.6, 63.5, 75.0, 81.0, 54.0, 57.0, 57.5, 57.7, and 82.2. A cellulose acetate membrane presents at least two difficulties; water evaporates very slowly from the treated cup; but more important, it is very difficult to free the system from imprisoned air, and perhaps impossible entirely to rid the system of the smaller bubbles.

So much success attended the trials with a copper-ferrocyanide precipitation membrane that the idea of making a hot-precipitation membrane was conceived. It is obvious that when heated a porous cup is expanded and the pores are larger than in the cool state. If a cup is then boiled in distilled water with suction to remove as much air as possible and is then filled with hot copper sulphate (2.5 gm. per liter) and suspended in a beaker of hot potassium ferrocyanide (2.1 gm. per liter), the whole being maintained in a hot-water bath for several hours, upon cooling the precipitated membrane must undergo a reorganization which would seemingly tend to make it more compact. Many trials with such a membrane seem to indicate that such is the case. Such a membrane is quite permeable with suction during the preliminary boiling to free the system from air, and water comes down the tube freely. On cooling, however, and making a trial test, such a membrane may be found to be quite impermeable. Enough experimentation has been done to indicate that there is a happy medium as to the amount of heat to apply during impregnation, and good results have attended a moderate application of heat during the process of precipitation. The method of hot-precipitation is worthy of further investigation.

Much time has been spent in trying to arrive at a more effective method of removing air from the system than is possible by the simple expedient of boiling and suction, especially in the case of using rather impermeable porous cups or membranes. Two-holed rubber stoppers were tried, a stop-cock, with and without funnel attached, being inserted in the second hole. Filling the funnel and tube with boiling water, it was thought that the system might thus be flushed out, the gas bubbles being forced out at the lower end of the capillary. This idea was soon abandoned. More trouble was experienced from gas bubbles than before. Solid glass rods were inserted in the hole. These could be withdrawn under water and reinserted. No great success was attained. Either the inrushing water column was sheared off at the instant of closing, thus creating a vapor

tension area which does not "heal," or the forcing in of the rod disengaged gas bubbles from the interior of the stopper, which were forced out by the compression. Metal pistons were also similarly inserted, by which means a pumping action could be set up, combined with agitation. Their chief effect was to loosen the stopper. On the other hand, several devices, mentioned presently, were more successful.

In all, about a hundred and twenty trials have been conducted, many with considerable success, and some were frankly failures. The best record obtained was made with a LIVINGSTON calibrated atmometer cup (no. 5–548, fifteen ×), boiled in weak NaOH and washed well, but otherwise untreated. The capillary tube was constructed from two fused, five-foot lengths of tubing, the upper end of which was bent into a U-shape, three and one-half inches high and four inches across, since it has been found that gas bubbles are more easily removed from gradual bends than from sharp angles. Preliminary to the fourth trial, the apparatus was boiled up for a half hour, with several periods during which the suction was released in the manner suggested by LIVINGSTON. The apparatus was allowed to cool, and on the next day full suction was applied, which was successfully withstood. The boiling pot was then heated to 60° C., and then quickly removed. Seventeen minutes afterward the column fell after reaching an uncorrected height of 150.0 cm., the barometer reading at the time being 73.9 cm. This indicates a pressure of more than two atmospheres. This same cup also gave other good records.

For maximum success with the ASKENASY demonstration, in addition to the precautions already suggested by LIVINGSTON, the following suggestions are made:

a. The mercury used in the lower reservoir should be pure and clean. As soon as it becomes glazed on top, it should be removed and washed before further use.

b. It is an advantage to have the end of the capillary tube relatively close to the surface of the mercury. If, in addition, the end of the tube is beveled up to the capillary, using a file and water lubricant, the removal of air is facilitated, especially in the case of less permeable porous cups and membranes.

c. The rubber stopper must fit tightly in the cup. This is best accomplished by first twisting it in, all parts being bathed with water. After this the stopper may be further inserted by a combined kneading in of the edges at the same time that a pushing thrust is used. When the stopper is well-seated, it may be slipped onto the upper end of the capillary tube, which should have been previously marked with a thread gauge to bring the end of the tube flush with the under side of the stopper. It is perhaps an

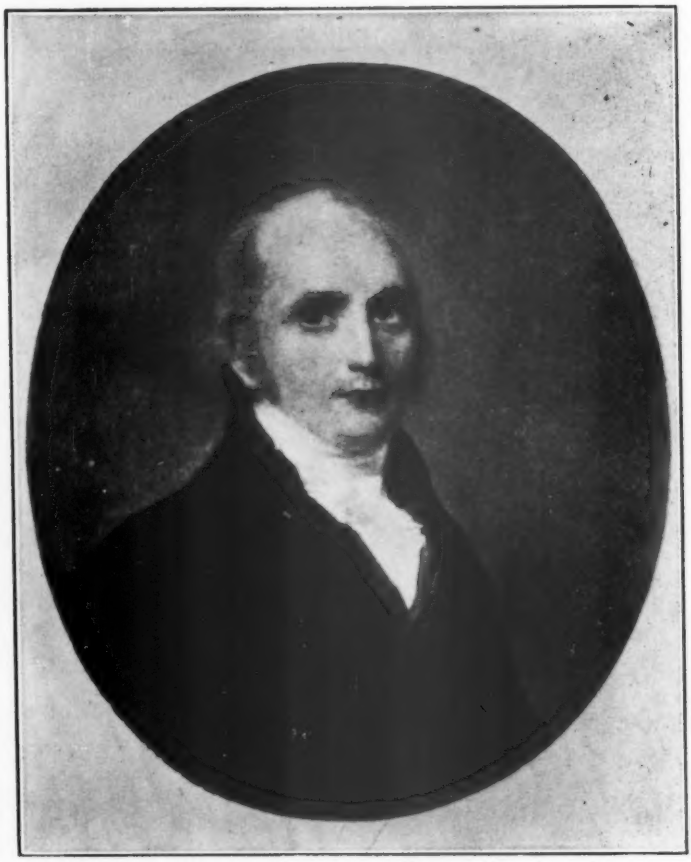
advantage previously to prepare the lower end of the stopper by cupping it with a sharp safety razor blade into a low cone, which gives little chance for air bubbles to lodge under the stopper.

d. There is no advantage, when using the ordinary porous cup, in long continued boiling, although this may not be true with treated cups which are difficult to free from confined air.

e. In making class-room demonstrations, it is best to try out the apparatus first by application of full suction, gradually applied and as gradually released. If the system does not break, the experiment is likely to be successful. Preliminary heating of the boiling pot to 60°–65° C. before beginning a trial allows the teacher to finish the demonstration in a few minutes' time, and the rise of the mercury column is so rapid that it is eagerly followed by the student. A convenient marker to record the progress made by the mercury is made from a short piece of rubber tubing, split, and slipped over the capillary tube. A little water between the surfaces in contact will furnish lubrication so that it may easily be moved. Jarring of the tube should be avoided, and may be largely prevented with proper rigid supports and ordinary care, not neglecting provision for expansion and contraction of parts.

This is a brief record of progress and difficulties. However, no teacher should be easily discouraged. With a suitable porous cup and ordinary laboratory facilities, the ASKENASY demonstration is not at all difficult to make; and it is hoped that it may be more generally employed in course work.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.



NICOLAS THÉODORE DE SAUSSURE
1767–1845

BRIEF PAPERS

NICOLAS THÉODORE DE SAUSSURE[1]

(WITH ONE PLATE AND THREE FIGURES)

NICOLAS THÉODORE DE SAUSSURE (plate IV) adopted quantitative methods in dealing with the problem of plant nutrition at a time when practically all other botanists were concerning themselves with theories of the vital forces which supplied food to plants in the form of the mysterious substance known as humus. DE SAUSSURE sustained and added to the discoveries of INGEN-HOUSZ and SENEBIER and proved that the green parts of plants take up and decompose a substance of the air at the same time that they assimilate water. His experiments on carbon assimilation are numerous and precise, and were presented with great perspicuity in his "Recherches Chimiques sur la Végétation" published in 1804. DE SAUSSURE found that the decomposition of carbon dioxide by green plant parts was a process necessary for the continued life and growth of the plant, but in addition he found that the carbon dioxide content of the atmosphere surrounding a plant might be increased, by artificial means, up to the point where it actually was injurious to the plant. Large quantities of carbon dioxide in the air were favorable adjuncts only if the plants were in condition to assimilate rapidly. After determining the amount of carbon appropriated by a plant, DE SAUSSURE found that the plant increased in weight by an amount out of proportion to the quantity of carbon fixed. Consequently, although he was of the opinion that the great mass of the vegetative body was built up from the components of the atmosphere, it was clear that a part of the vegetable mass was derived from the fixation or utilization of the soil solution.

The family of DE SAUSSURE was of French origin but about the middle of the sixteenth century they were among the religious refugees who fled from France to the little Republic of Geneva. Members of the family became prominent in the governmental affairs of the republic, and even at the present time some of its members are citizens of Geneva. The DE SAUSSURES have been interested in scientific pursuits as well as in the course of government. NICOLAS, the grandfather of NICOLAS THÉODORE, was a country gentleman engaged in the management of his various estates and interested in agricultural matters. He published several practical treatises; on methods of cultivation, pruning, crop failures, and soil fertility. His son, HORACE BENEDICT, who was the father of NICOLAS THÉODORE, was a scientist also

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station as Paper no. 219 of the Miscellaneous Series.

and we are indebted to him for many of the data on which the very foundations of geology rest. HORACE BENEDICT DE SAUSSURE, figure 1, was one of the first and most successful Alpine travellers: perhaps he was also one of

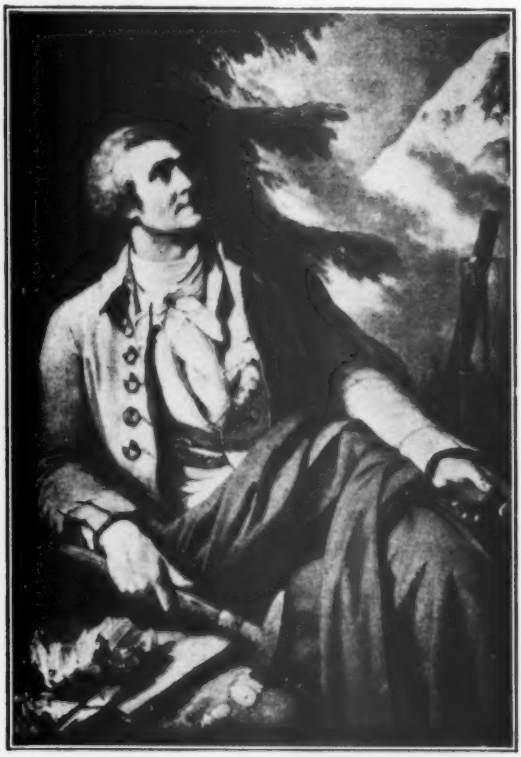


FIG. 1. HORACE BENEDICT DE SAUSSURE, father of NICOLAS THÉODORE DE SAUSSURE

the most unique, for he undertook his journeys for the sake of studying geology, physics, and other natural phenomena as well as for pleasure. Mountain chains, rocks, streams, glaciers, and the atmosphere were studied so diligently that even today a large share of his work remains undisputed. He must have been ingenious as well as diligent, for he invented or perfected a number of instruments which were required for the physical measurements in his work. With such a background and such an environment it is not surprising that NICOLAS THÉODORE turned his thoughts to scientific studies. Much of the life of NICOLAS THÉODORE DE SAUSSURE may be found in "The life of HORACE BENEDICT DE SAUSSURE" by DAVID FRESHFIELD.

We are indebted to FRESHFIELD for the excellent portrait in Plate IV, and for figure 2 which indicates the material environment of NICOLAS THÉODORE during his early life.

NICOLAS THÉODORE, the eldest son of HORACE BENEDICT DE SAUSSURE, was born at Geneva, October 14, 1767. The boy was educated at home for a time because his father disapproved of the educational methods of the public schools. Later, however, he attended l'Académie de Genève, became very much interested in the natural sciences, and finally became his father's assistant and companion. After his father's journeys ceased, NICOLAS THÉODORE found time for his own botanical and chemical researches. The family fortunes were sufficient to permit considerable travelling so that NICOLAS

FIG. 2. The town house in Geneva where NICOLAS THÉODORE DE SAUSSURE spent much of his childhood.

THÉODORE, as well as his father, was in direct contact with the work of foreign scientists. The summer of 1793 he spent in England in company with distant relatives, but late in December of that year his mother informed him of the loss of the family fortune and advised him to seek a travelling tutorship, for his father was no longer able to provide him with the ease to which he had been accustomed. Such a tutorship was not to be found, however, for the English patrons were far more interested in the classics than in scientific studies, and NICOLAS THÉODORE returned to Geneva in the summer of 1794. He had not been home long when he and his brother were forced to flee to Rolle on account of the great disorder in Geneva resulting from the effects of the revolution in France. It was in Rolle in 1794 and 1795

that NICOLAS THÉODORE spent a great deal of time correcting proof of the last two volumes of his father's "Voyages." The following year he returned to Geneva, married RENÉE FABRI, and settled down once more to his scientific career. The treatise on carbonic acid in its relation to vegetation,

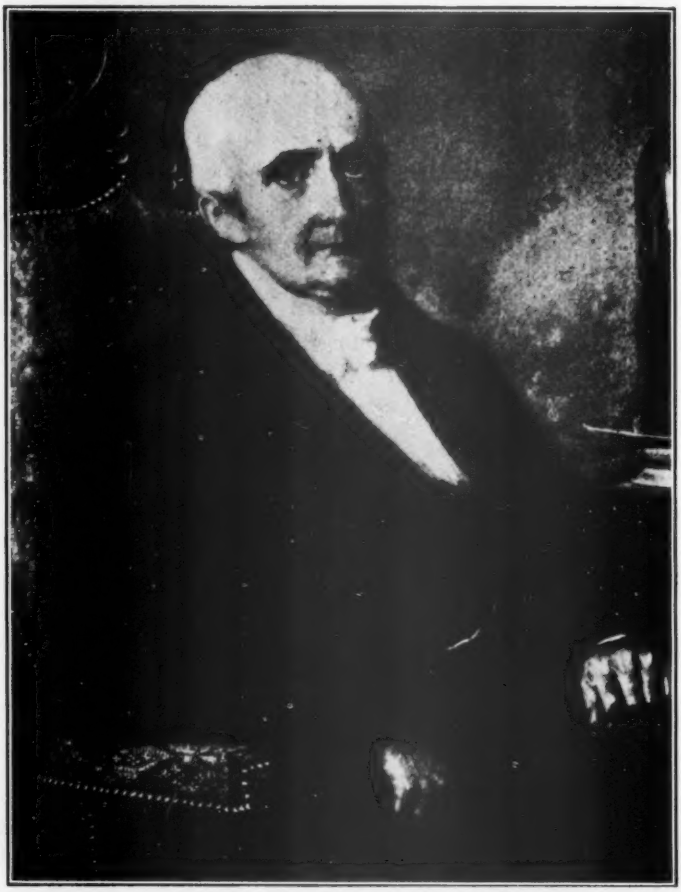


FIG. 3. NICOLAS THÉODORE DE SAUSSURE in later life as Professor of Mineralogy and Geology in the Academy of Geneva.

a prelude to his principal work "Recherches Chimiques," was written in 1797. After a short interval there was more travelling so that several years were spent in England and in France, and the wanderers did not return to

Geneva until 1802. At that time DE SAUSSURE received the appointment of Professor of Mineralogy and Geology in the Academy of Geneva. The position carried a stipend but was an honorary appointment with no regular duties; thus he was free to continue the work he had begun. His "Recherches Chimiques sur la Végétation," which was published in 1804, received great attention from the scientific world, but it was a long time before his ideas were completely understood and appreciated. The old humus theory had such a strong hold on the botanists of Europe that DE SAUSSURE'S experiments were misconstrued and misunderstood until the reaction against the theory of a vital force set in twenty or thirty years after his publication.

DE SAUSSURE insisted that certain minerals found in the ash of plants were not accidental ingredients taken in with the soil water but were essential to the plant as foods in spite of the fact that they were often present in extremely small quantities. He recognized the fact that in the absence of any one of those essential elements development of the plant was impossible. He also was aware of the fact that the amount of an element was not the all-important consideration, but that a trace of one substance might be sufficient while larger quantities of other substances were needed.

Respiration was studied as well as carbon assimilation. Growth of the plants was impossible without respiration, and those parts in which physiological processes were most active required more oxygen than the parts in less active states.

NICOLAS THÉODORE DE SAUSSURE was a corresponding member of the French Institute and a foreign member of the British Royal Society. He was also a member of the Société de Physique et d'Histoire Naturelle of Geneva. Figure 3 is taken from a portrait painted during his later life. Most of his life was spent in Geneva and it was there that he died April 18, 1845. With INGEN-HOUSZ and SENEBIER, DE SAUSSURE is responsible for founding the modern theory of plant nutrition.—HELEN HART, *Division of Plant Pathology and Botany, University Farm, St. Paul, Minnesota.*

SOME ACCESSORIES FOR THE DISSECTING MICROSCOPE

(WITH ONE FIGURE)

The use of the Greenough type of binocular dissecting microscope may be greatly facilitated by the addition of the attachments described below.

FIG. 1. Some accessories for the dissecting microscope.

In this arrangement the microscope, a Leitz UBM, is mounted on a table by means of a clamp stand *P*, placed in front of the operator. By reversing the position of the stand and placing it on the opposite side of the micro-

scope from the operator even more working space can be obtained. The focusing of the instrument while dissecting is accomplished by the foot-operated mechanism shown. A shaft, fitted with ball bearings mounted in block *A*, has a pulley *B* on one end and is connected to the focusing wheel of the microscope by a rod with leather universal joints at *C* and *D*. The mounting for the wheel *E* is bolted to the table and the pulley *F* is belted to *B*. By having a tight belt and closely fitted universal joints lost motion is eliminated and the operator may focus the microscope as easily and as accurately as when the hand is used.

The proper illumination for dissection requires a beam of light low in heat. The lamps *G* and *H*, shown in the picture, are satisfactory. Osram 6 volt, 25 watt, Nitra globes supply the light, current coming from a transformer on the lighting circuit. An ordinary socket *I* holds the globe in position in a metal cylinder *J*, $2\frac{1}{8}$ inches in diameter. In front of the globe is a condenser composed of two lenses of two inch diameter and two inch focal length. The lenses are mounted in a sliding sleeve and are adjusted by moving the handle *K* attached to the sleeve and projecting through a spiral slot in the cylinder *J*. A cone-shaped nosepiece *L* to hold filters fits over the lower end of the cylinder. This lamp may be adapted for use with the compound microscope by using a ground glass filter.

For dissection work three lamps are used, two mounted either together or separately provide illumination from above, and one from below. This lower lamp which does not show in the picture is directed upward through a hole in the table top. Three small switches *Q* control the three lamps independently. A diaphragm set flush with the table top above the lower lamp regulates the size of field and aids in centering the object.

A mechanical stage *M* adapted to hold a petri dish provides for accurate movement of the object which is held in place by suitable spring wire clamps *N* and *O*. Dissection may be done in air or in water. A suitable shade around the oculars gives a brighter field and prevents eyestrain. This equipment has proved helpful in studying structural relationships in plants. It should also aid in microchemical testing and other work requiring manipulation of material while it is under observation.—A. S. Crafts, *Division of Plant Pathology, University of California, Berkeley.*

EFFECT OF SOMATIC INJURY UPON YIELD IN CORN[1]

Introduction

In a series of experiments on hens the author (**5, 6**) found the partial removal or mutilation of the ovary or merely bodily injury produced in some birds a stimulating effect in egg production. In approximately an equal number of birds in the same experiments the operation produced a retarding effect. When the operations were performed on six-weeks' old pullets,[2] similar stimulation and retardation were noted in the length of time until the first egg was laid.

In order to test for a similar reaction in plants a series of experiments was performed on corn. While the results do not warrant a conclusion directly related to the experiments on animals such as those described above and those of ZELENY (**7**), PEARL (**4**), HARTMAN (**3**), and others, yet some very interesting results from an economic point of view are indicated by the experiments. The disputes arising from some appraisals by insurance companies on hail or wind injured crops, the custom in some localities of allowing lambs to eat off the lower leaves of corn, seem to the author to be examples to which the results of this experiment may be related.

The author wishes to express his appreciation to Mr. SAM DUNKELBERGER, Halstead, Kansas, who harvested the corn in the fall and on whose farm the experiments were performed during the summer of 1929, also to his colleague, Dr. E. C. DRIVER of Smith College, for helpful criticisms during the writing of this paper.

Methods

The experimental plants were selected in a field of about four acres in size. The corn was between five and six feet tall and the silk was just beginning to appear when the experiments were begun. The plants of each experiment were tagged with different sun-fast cloth labels.

Each experiment, with its results is described below.

Results

EXPERIMENT 1.—A search was made to find 50 hills each containing 2 plants of equal height, thickness and quality. One stalk was to be the operated and the remaining one the control.[3] In the "operated" stalk 3 longitudinal incisions, about 3 inches long, were made through the stem at about 18 inches from the ground. The incisions were made from different sides of the stalk.

[1] Contribution from the Department of Zoology, Smith College, no. 164.

[2] Paper now in manuscript form.

[3] In case there was a slight advantage in one of the two stalks it was always given to the control. This procedure holds for the other experiments as well.

There were 45 hills recovered in November when the corn was harvested. The 45 operated stalks produced 48 ears which yielded 25 pounds of shelled corn. The 45 control stalks produced 45 ears of corn which weighed 25 pounds and 3 ounces when shelled. Obviously there is no significant difference in total corn yield in this experiment. During the summer it was noticed that there were several operated stalks considerably taller than their particular controls, yet this factor was not critically analyzed.

EXPERIMENT 2.—The material for this experiment was 25 hills each containing 2 similar plants. From one plant in each hill all the leaves were removed except the two nearest the tassel. These were called "operated plants" and their corn yield was compared with the control plants. The 23 operated stalks recovered carried 7 ears of corn which produced 1 pound 4 ounces of shelled corn as compared with 29 ears from 23 control stalks, which yielded 14 pounds and 5 ounces of shelled corn.

EXPERIMENT 3.—This experiment was similar in every way to experiment 2 except that every other blade was removed from the operated plant. There were 22 operated stalks recovered on which there were 19 ears which produced 5 pounds 10 ounces of shelled corn. From the 22 control stalks, 23 ears were obtained, the shelled corn of which weighed 12 pounds and 12 ounces.

EXPERIMENT 4.—Fifty individual plants were selected on which there were either 2 or 4 ears. In case there were 2, one of them was cut, directly down the middle, leaving only the back part of the husk to hold the ear somewhat intact. If there were 4 ears on the stalk every other one was cut. The uppermost ear on each plant was used alternately as a control and an operated ear. A few days after the operation the operated ears spread out, due to the lack of tension, causing the silk to appear through the incision. Soon the cob began to curl and the immature kernels became hardened by the weather. Many of these ears became so infected with molds that they were lost entirely.

There were 30 operated ears recovered which produced 2 pounds 12 ounces of shelled corn as compared with 39 control ears which yielded 20 pounds and 8 ounces of shelled corn.

The data described in these experiments are shown also on table I.

Discussion

Part of a letter from a farmer, who was acquainted with these results, appears below.

"In August we had a hail storm, when the ears of corn were partly made; the grain had already started on the cob. During the storm the blades were knocked off but the ears still remained. The insurance adjuster refused to grant my claim, saying that no damage was done to the actual

TABLE I

NUMBER OF EARS AND THE WEIGHT OF SHELLED CORN OBTAINED IN FOUR EXPERIMENTS ON THE EFFECT OF INJURY ON CORN YIELD

EXPERIMENT	NO. OF EARS		SHELLED CORN		YIELD PER EAR	
	OPERATED	CONTROL	OPERATED	CONTROL	OPERATED	CONTROL
			lbs.	*lbs.*	*lbs.*	*lbs.*
1. Incisions through corn stalk	48	45	25.00	25.19	0.52	0.56
2. Every blade removed	7	29	1.25	14.31	0.18	0.49
3. Every other blade removed	19	23	5.63	12.75	0.30	0.55
4. Ear cut longitudinally	30	39	2.75	20.50	0.09	0.53

corn yield. The yield was actually lessened, and by your experiments I can prove my claim." It is obvious from table I that the more blades removed before the corn is ripe the greater is the damage. Some Iowa farmers allow lambs to eat off the lower leaves of corn. This experiment may have a bearing on this practice as well.

Although the yield was less in the operated plants, the results may still be related to the zoological experiments cited in the introduction. It was mentioned there that both a stimulation and retardation was noted. Perhaps in the work on corn only the retardation became evident. Some plants, *e.g.*, dandelions or willows, might experience some stimulation to a more luxuriant growth of leaves or of seed, when injured.

Conclusion

Corn plants which have been injured produce less corn than uninjured plants.—MORRIS STEGGERDA, *Smith College.*

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NOTES

The Annual Election of Officers.—During the last two months the annual election of officers of the American Society of Plant Physiologists has been in progress. The Secretary-Treasurer, Dr. H. R. Kraybill, of Purdue University, has just announced the results of the balloting, as follows:

President, Dr. H. R. KRAYBILL, Purdue University.

Vice-President, Dr. W. E. TOTTINGHAM, University of Wisconsin.

Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute.

The year just closing has seen steady growth of the Society, and the new officers should find conditions pointing to a bright future. With the cooperation of the present membership it should be relatively easy to bring the membership to the 500 mark within the next year.

Committee on Physical Methods.—A committee has been organized to do for physical methods of research a service similar to that rendered by the Committee on Analytical Methods for the chemical methods of attack. It will be the purpose of the committee to summarize and possibly to recommend standard methods of procedure for biophysical research. The chairman of the committee is Dr. R. B. HARVEY, of Minnesota. The other members so far chosen are Dr. G. W. SCARTH, McGill, who will compile methods of protoplasmic research; Dr. L. O. REGEIMBAL, Minnesota, pH and conductivity measurements; Dr. D. R. HOAGLAND, California, nutrient solution methods. Dr. E. S. JOHNSTON, Maryland, osmotic pressure and general water relations; Dr. H. W. POPP, Pennsylvania State College, light. Other members may be added later, as the work of the committee develops. There is opportunity for a real service to plant physiology in this project, and the results of the work of collating methods will be awaited with much interest.

Life Membership Committee.—President KRAYBILL has asked the following members to serve on the CHARLES REID BARNES Life Membership Committee: Dr. S. F. TRELEASE, chairman; Dr. R. B. HARVEY, Dr. J. P. BENNETT, Dr. L. F. GRABER, and Dr. D. B. ANDERSON. The duty of selecting the sixth recipient of this honor will be the main function of the committee. The results of the selection in past years have been announced at the annual dinner of the physiologists, and a similar arrangement may be made for the Cleveland meeting in December, 1930.

The Purdue Section.—The Purdue Section of the American Society of Plant Physiologists has had a splendid year. With 31 members, 15 of

whom are members of the national organization, the average attendance has been about 25 at each biweekly meeting. It has been customary to have invited speakers at the first and last meetings of the year. Dr. E. J. KRAUS, of the University of Chicago was present at the first meeting giving a very helpful informal discussion of plant research. The speaker at the final meeting, was Dr. W. E. TOTTINGHAM of Wisconsin, who discussed the problems of temperature and metabolism, and led a discussion of the factors affecting winter hardiness after the lecture.

The programs for the regular meetings were as follows:

October 7, Crop nutrition studies in Europe, S. D. CONNER.

October 21, Physiology of the apple blotch fungus, E. J. KOHL.

November 4, Further studies on color and quality of tomatoes, J. H. MACGILLIVRAY.

November 18, Purification and properties of tomato mosaic virus, P. H. BREWER.

December 2, Recent respiration studies at the Cambridge Botany School laboratory, R. E. GIRTON.

January 13, Joint evening meeting with the Biological Society devoted to reports of the Des Moines meeting of the A. A. A. S.

February 3, The Neubauer method of soil analysis, S. F. THORNTON.

February 17, Fungus inhibition and staling, C. L. PORTER.

March 3, Some recent results of the corn stalk method for testing soil fertility, G. N. HOFFER.

March 17, Ozone treatment of fruit in storage, C. E. BAKER.

At the final meeting, the officers of the Section for the next year were installed. Dr. R. E. GIRTON is the chairman for 1930–31, and S. F. THORNTON, secretary. The Purdue Section has set a high standard for its activities, and deserves the fine success which has attended it.

The Minnesota Section.—The Minnesota Section also reports some excellent meetings on hardiness, light, etc. The April meeting occurred on April 15, and in addition to an address by Dr. CHARLES SHEARD, of the Mayo Clinic, on "Physiological effects of irradiation," several demonstrations of apparatus were made. Mr. HAROLD MITCHELL demonstrated the Illuminometer method of light measurement; Dr. L. O. REGEIMBAL demonstrated and discussed the Potentiometer Light Recorder, which uses General Electric Photoelectric Cells, and Dr. H. SHIRLEY demonstrated and discussed his new Radiometer. Dr. C. H. BAILEY is chairman of the Section, and R. B. HARVEY, Secretary.

Deaths of members.—We regret to announce the death of two of our members.

Patrick Beveridge Kennedy.—The death of PATRICK BEVERIDGE KENNEDY, Professor of Agronomy at the University of California, occurred on January 18, 1930. Born at Mt. Vernon, near Glasgow, Scotland, in 1874, he grew up with a love for agriculture, horticulture and botany, and began his education along these lines in Scotland and England before he came to America. He attended the Ontario Agricultural College, and finally received his first degree from the University of Toronto in 1894. After spending a short time at the Ontario Agricultural College as assistant chemist, he went to Cornell, where in 1899 he won his Ph.D. degree. He was in the Department of Agriculture for a couple years, and then went to Nevada, where he was connected with the work in botany, horticulture and forestry from 1900 to 1913. In 1914 he went to the University of California, where his lofty ideals of research and his rare love and understanding of the plant materials with which he worked attracted ever increasing numbers of graduate students, who found with him unequaled opportunities for study in the field of agrostology.

He spent much time on the taxonomic revision of the genus *Trifolium*, and was working on the section of the bladder clovers at the time of his death. Many monographs were written on grasses, saltbushes, forage plants, alpine plants, etc., and he was interested in the introduction of new grasses to the stock ranges of California. Harding grass, Kikuyu grass, Rhodes grass, and bulbous blue grass were among those brought in to improve the ranges. He was an authority on turfs, and his advice was widely sought in the management of greens on country club grounds. To his fellow members of the American Society of Plant Physiologists, he is best known for his work on the perplexing problem of control of the wild morning-glory by chemical sprays.

In spite of domestic cares, he was always cheerful, and his willingness to serve others endeared him to all who knew him. One of his greatest pleasures was the loyal friendship of his students and colleagues.

Grace Barkley.—The death of Dr. GRACE BARKLEY occurred on April 1, 1930. Death was believed to have resulted from a cerebral hemorrhage following a fall down a stairway. Although she lived for six days after the fall, she was never able to explain the circumstances.

She was completing her third year at DePauw University, where she had gone after receiving her Ph.D. at Chicago. Her main work was on the origin of spiral markings in the protoxylem of *Trichosanthes*. She had previously published on the origin and development of secondary wood in *Yucca*.

New Laboratory at the University of Chicago.—The work in plant pathology and plant physiology at the University of Chicago is moving into the new laboratory on Ingleside Avenue, which has been erected during the last six months in proximity to the new greenhouses which were built during the preceding summer. The laboratory is equipped for many different lines of work. Pathology has a general laboratory, research laboratory, refrigeration room, inoculation room which can be sprinkled down, and a preparation room. In addition it shares use of other rooms, as store room, apparatus room, etc. Plant physiology has a laboratory for elementary and general class work, a biochemical laboratory with adjacent balance room, nitrogen laboratory, biophysics laboratory, seed laboratory, microchemical laboratory, and radiation laboratory for ultra-violet, X-ray, and other short wave radiation research. There is also an oven room, and dark room for any work requiring dark conditions. There is also a well equipped photographic room and dark room, providing facilities for every research requirement along photographic lines.

Temperature control rooms are located in the basement, with a possibility of $-40°$ C. at the lowest. A grinding room will be developed in the basement, and soil centrifuge for work in soil physics.

The greenhouses are also provided with temperature and humidity control chambers, and soil temperature control apparatus. These new laboratories offer unique opportunities for investigation in plant physiology and plant pathology. As a plant of this sort is appreciated more when seen in operation, investigators passing through Chicago are invited to see the new plant. It will be ready for complete occupation by the first of October. Classes are using the instructional laboratories this summer.

Statistical Methods for Research Workers.—The third edition of R. A. FISHER's monograph on "Statistical Methods for Research Workers" has come out after the second edition was only 2 years old. Naturally the treatment of statistics does not change rapidly enough to warrant frequent editions, and the multiplication of editions without sufficient reason ought to be discouraged. Two new subsections, comprising about 10 pages, are added to section 57. These new paragraphs deal with fragmentary data, and the amount of information, design, and precision; they illustrate some wider applications of the method of maximum likelihood, and the quantitative evaluation of information. The author has also added a section of citations of his own papers, whether pertinent or not. These changes indicate not sufficient reason for a new edition. The price of the book is 15 shillings, and orders should be sent to Oliver and Boyd, London.

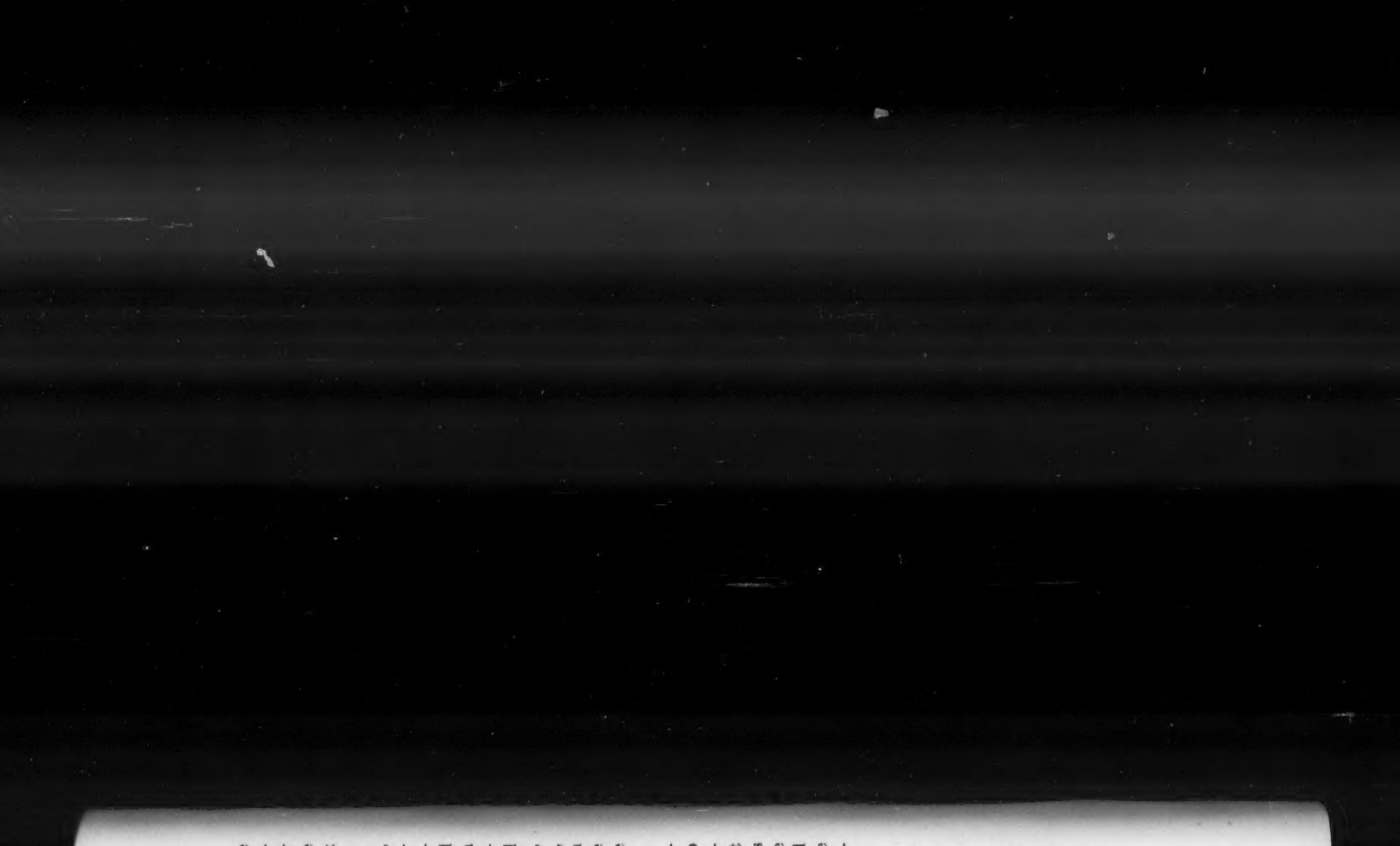
General Physiology.—A textbook of general physiology suitable for beginners has been prepared by Dr. G. W. SCARTH, and Professor F. E.

LLOYD, of McGill University. The text (194 pages) is accompanied by laboratory exercises, which occupy 60 pages in the back of the volume. There are 14 chapters, with the following headings: Life as a mechanism; organization of protoplasm; surface tension in physical systems; surface tension in cells; adsorption in physical systems; adsorption in cells; diffusion and osmosis in physical systems; diffusion and osmosis in cells; ions and their determination in physical systems; ions, particularly H-ions, and their determinations in cells; electric potential and electric currents in physical (non-metallic) systems; electric potential and electric currents in cells; colloids in physical systems; colloids in the cell. The laboratory outlines are arranged in 13 groups, the first eleven of which cover the ground of chapters 2–14. The last two outlines deal with ultramicroscopy, and the general properties of enzymes. The treatment is simple, and should furnish a stimulating introduction to the field it covers. Naturally there are a number of topics one might expect to find in a general physiology which have been omitted. Nevertheless, the text will offer many good suggestions for class experiments along lines that never have been emphasized sufficiently in teaching plant physiology and general physiology. The price quoted for the text is $2.75, and the publishers are John Wiley and Sons, New York.

New Tables for Reducing Sugars.—A little volume of Recalculated Tables for the Determination of Reducing Sugars by Bertrand's Method has been published by the author, Dr. Z. I. KERTESZ, of the Department of Agricultural Chemistry, the Agricultural Experiment Station, Geneva, N. Y. It gives in both English and German, complete and exact directions for the Bertrand method for reducing sugars. It should be very useful for the non-chemist as well as the chemist. In the original tables the even increments were in terms of sugar. Here they are for Cu, from 1.0 to 190.0 mg., by 0.2 mg. increments, making the tables far simpler to use. The sugars include glucose, invert sugar, maltose, lactose, mannose, galactose, sorbose, arabinose and xylose. This method is commonly in use in Europe and is becoming more and more favored in this country. The volume contains 34 pages, and can be purchased for 75 cents, from the author.

Exploring for Plants.—Although not specially useful to plant physiologists the editor cannot refrain from mentioning two books which have recently come to his notice. One of these, "Exploring for Plants," is by DAVID FAIRCHILD, who has for many years explored the earth for useful plants. This interesting book is a record of the Allison Vincent Armour expeditions for the Department of Agriculture during 1925, 1926, and 1927.

The life of a botanist is full of romance, and this story by FAIRCHILD is one of the romances that everyone will want to enjoy. It is told in 43 captivating chapters, and beautifully illustrated. For $5.00 one can enjoy one of the most interesting botanical stories of the twentieth century. The publishers, Macmillan Co., New York, have done an excellent piece of work in the make up.

Rock Gardens and Alpine Plants.—The other work referred to above, is HENRY CORREVON'S "Rock Garden and Alpine Plants," also from Macmillan. HENRI CORREVON is the greatest living student of alpine vegetation. His first book on the subject was published in 1884, and he has had a constant interest in the subject for more than 50 years. The book is a storehouse of information for anyone interested in rockeries, and the problems of acclimatisation and cultivation of mountain plants in lowland gardens. There are 15 illustrations, eight of which are colored plates, one thinks not any too true to the natural colors. If for no other reason, one should read it for the glimpse it gives into the romantic interest in plants in the life of its distinguished author, HENRI CORREVON. The quoted price of this work is $6.00, and orders should be sent to the publishers.

Physiology and Biochemistry of Bacteria.—Received too late for extended notice in this number of PLANT PHYSIOLOGY, we call attention to the publication of the last two volumes of this valuable work on the physiology of the bacteria by R. E. BUCHANAN and E. I. FULMER. The first volume was noticed in the April number of this journal, 1929. The set as a whole represents an enormous amount of work, and the authors have made a permanently valuable contribution to our knowledge of bacterial life. A more extended notice will be given in the October number. Those who desire the complete work should address Williams and Wilkins, Baltimore, Maryland. The last two volumes are $7.50 each.